

TITLE: UNIVERSAL PRIMERS FOR WILDLIFE IDENTIFICATION**FIELD OF THE INVENTION**

The invention relates to the identification of novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin at species and sub-species sources. The invention also provides a method for the identification of fragments on mitochondrial cytochrome b gene in biological material of unknown origin.

BACKGROUND & PRIOR ART REFERENCES

A large number of studies in evolutionary biology utilize phylogenetic information obtained from mitochondrial cytochrome b gene. It has been identified a potent molecule to distinguish the phylogenetic depth of different lineages to family, genus and species in molecular taxonomy¹⁻⁶⁶. A vast database of the sequences of cytochrome b gene of different animal species has accumulated in public databases such as GenBank, NCBI (<http://www.ncbi.nlm.nih.gov>) etc. We have utilized this capacity of cytochrome b gene in establishing the identity of the origin of animal parts and product to its family, genus and species sources. The technique developed is based on a pair of universal primer that can amplify a small fragment of cytochrome b gene from a vast range of animal species.

Establishing identity of confiscated animal parts and products is a great challenge to law enforcement agencies because none of the methods available till date is too efficient to reveal the identity of animal remains beyond a reasonable doubt. Morphological markers, described for certain species allow the identification of complete specimen of animals⁶⁷. However, a complete specimen is confiscated very rarely by the investigation agencies; therefore, these marker are not practical in wildlife forensics. The biochemical traits such as the bile characteristics⁶⁸ blood heam analysis^{69,70} etc. have also been employed in wildlife forensic for identification of individual species. The difficulty of these markers are that these markers are limited in number and are rarely found in their natural forms in which these were originally described as the characteristic of a particular species.

The molecular approaches such as micro-satellite based identification⁷¹, Restriction fragment length polymorphism analysis of mitochondrial genes or PCR based species specific STS markers require the prior information of the species to establish the

identity^{72,73}. These methods also need a significant amount of DNA material to be analysed. We may not have the prior information about the species origin of confiscated animal parts and product in forensics, therefore, these methods are not really useful and practical in wildlife identification. The technique invented by us is universal, therefore does not require any background information to establish the identity of any unknown confiscated remains at family, genus and species sources. Being a PCR based procedure it can be applied with trace amount of any biological material. Because the amplicon length is small (472 bp); therefore, it can work perfectly with the mutilated remains, which are commonly seized by the crime investigation agencies. It does not require the large amount of genetic material i.e. DNA to be analyzed to establish the identity, hence, can detect a minute amount of adulteration in food products. The procedure described is simple and very fast. Due to the said advantages, the procedure invented by us is most suited for forensic wildlife identification.

OBJECTS OF THE INVENTION

The main object of the invention is to identify a fragment on mitochondrial cytochrome b gene capable of significantly discriminating among various evolutionary lineages of different animal species.

Another object is to identify a fragment on mitochondrial cytochrome b gene which is flanked by the highly conserved sequences at a vast range of animal species.

Yet another object is to detect a fragment on mitochondrial cytochrome b gene which is polymorphic inter-specifically, but monomorphic at intra species sources.

Still another object is to develop the universal primers to amplify the fragment on mitochondrial cytochrome b gene using polymerase chain reaction.

Another object is to develop a PCR protocol that works universally with DNA template of any unknown origin (i.e. all the animal species).

Yet another object is to provide a universal method for identification of species of analyzed material (i.e. the DNA isolated from confiscated animal remain of unknown origin) using the public databases such as GenBank, NCBI etc.

Still another object is to provide a universal method of animal identification to establish the crime with the criminal beyond a reasonable doubt.

Another object is provide a universal method to establish the identity of biological materials such as skin, horns etc confiscated from animal poachers, if it is that of an endangered species.

Yet another object is to provide a universal method for establishment of the identity of confiscated animal parts and products of endangered animal species for the purpose of production of molecular evidence of animal hunting and related crime in the court of law, so that the human violation to the wildlife resources could be controlled.

Still another object is to provide a universal technique to have an idea of the geographical location of the commitment of wildlife crime based on the haplotype of poached animal identified by the universal primer invented.

Another object is to provide a universal technique of animal identification to detect the adulteration of animal meat/products in vegetarian food product for the purpose of food fortification, by the food fortification agencies.

Yet another object is to provide a universal technique for detection of the origin of blood or blood stains etc collected from the scene of crime related to offences such as murder, rape etc, in order to establish the origin of blood found at scene of crime when it sounds as if criminals have wontedly spread the blood of an animal at the scene of crime, to confuse the crime investigation agencies and forensic scientists with human blood.

Another object is to invent and authenticate a universal technique that can be converted to a (a) 'MOLECULAR KIT' and (b) 'DNA CHIPS' based application to meet the requirements of above objectives.

SUMMARY OF THE INVENTION

Accordingly, the invention provides novel universal primers that can amplify the fragment of cytochrome b gene of any animal species in polymerase chain reaction (PCR) and reveal the identity of the biological material of any unknown animal origin

DETAILED DESCRIPTION OF THE INVENTION

Keeping in view the above objectives, the cytochrome b gene sequences (1140 bp) of 221 distantly related animal species (listed in Table 1) representing various families were obtained from public database NCBI (<http://www.ncbi.nlm.nih.gov>). These sequences were aligned using the software *Clustal X*(1.8)(NCBI, USA) and a fragment (of 472 bp, alignment shown in Table 2) of gene was identified which had all the features mentioned above under column 1, 2 and 3 of sub-heading 'Objectives of invention'. As for the identity of this fragment we would like to mention that it includes the nucleotides between 398 to 869 in *Antelope cervicapra* and *Felis catus*; however, 399 to 870 in *Homo sapiens sapiens* species. Except at few positions (marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, giving rise to their unique molecular signature. These molecular signatures are characteristic of its species and form the basis of revealing the identity of the biological material of an unknown animal origin by the procedure invented by us. Considering *Antelope cervicapra* as a representative species, the sequence of this fragment is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antelope cervicapra*:

“taccatgaggacaaatatcttttgaggagcaacagtcacccaatctccttcagcaatccatacatcggtacaaacctagtaga
atgaatctgaggagggttctcagtagataaagcaacccttaccgatttttgccttccactttatcctcccattatcattgcagccctta
ccatagtacacctactgttttccacgaaacaggatccaacaacccacaggaatctcatcagacgcagacaaaattccattccaccc
ctactacactatcaaagatctcctaggagctctactattaatttaaccctcatgcttctagtctattctcaccggacctgcttgagacc
cagacaactataccagcaaaccacttaatacacccccacatatcaagccgaatgatacttctatttgcatacgcaatcctccga
tcaattcctaacaaactaggagg”

A pair of universal primer was designed to amplify this fragment in polymerase chain reaction (PCR). These primers were named as 'mcb398' and 'mcb869' because of its property to amplify a region of mitochondrial cytochrome b gene between nucleotides 398 to 869 of *Antelope cervicapra*, a representative animal species for this invention. We took

this animal species as representative species because the idea of developing such a novel primers came in the mind of inventors while they were working on the genome of this animal in Centre for Cellular and Molecular Biology, Hyderabad, India. These primers work universally because its 3' end are highly conserved amongst a vast range of animal species (shown in Table 2). As mentioned above, the DNA fragment (sequence of which is shown above) targeted by these primers is highly polymorphic inter-specifically; however, it is monomorphic among the individual of same species (Tables 6, 7a, 7b, 7c, 7d and 8, respectively). These unique features of the targeted region enable these primers to generate the molecular signatures of an individual species; thereby, enabling them to differentiate amongst the animals of different species (see in Figure 1c). The variation within the fragment amplified by these primers increase with increasing distances of evolutionary lineages of two animals (Table 8). These unique features of the fragment amplified by the universal primers 'mcb398' and 'mcb869' invented by the applicants fulfill the objectives of invention.

Thus, the primers invented by us can generate the molecular signature from any biological material of unknown animal origin, which actually is the characteristic of its family, genus and more precisely, the species. When these signatures are compared *in-silico* with the signatures already available in public databases (viz., GenBank, NCBI database etc) using 'BLAST software'⁷³, it indicates identity of the family, genus or species of the analyzed material, which in turn is confirmed practically by comparing with the reference animals of the revealed family, genus or species, by including them in the further analysis by the primers 'mcb398' and 'mcb869'. The complete procedure involved in the *analyses* (the word, '*analyses*' should be understood with the stepwise procedure to establish the identity of the biological remain of any unknown animal origin for the aims mentioned in columns 1-13 under sub-heading 'Objectives of invention') is briefed under 'Examples 5 and 6, respectively, as well as illustrated in Figures 1a, 1b and 1c, respectively.

BRIEF DESCRIPTION OF DRAWING AND TABLES

Figure 1a. Illustration of the step-wise procedure involved in *analyses*. The unknown biological material i.e. 'adil.flesh' refers to the confiscated skin mentioned in 'Example 6'. The arrow marks indicate the stepwise procedure involved. The brief description of Figure 1a is as follows:

The biological material i.e. the confiscated skin 'adil.flesh' was subjected to DNA isolation using the standard procedures⁷⁴. The DNA obtained was amplified using the primers 'mcb398' and 'mcb869' in PCR, fractionated in 2% (w/v) agarose gel, visualized and photographed under UV light using Gel Documentation System (Syngene, USA). The lane 'M' shown in the photograph represents the molecular weight marker (Marker XIII, Boehringer mannheim). Lane 1 shows the PCR amplicon (472 bp) obtained from 'adil.flesh' using primers 'mcb398' and 'mcb869'. The PCR amplicon obtained were sequenced at both the strand using "ABI Prism 3700 DNA Analyzes, PE-Applied Biosystems). The chromatogram shows the sequences (about 80 bp long, i.e. between 150-230 bp of sequence (328 bp), revealed from the PCR product of 472 bp length) obtained from 'adil.flesh'.

Figure 1b. Illustrates the further steps involved in *analyses*. The sequence (328 bp) revealed from 'adil.flesh' was subjected to homology search in *nr* (i.e. *non-redundant*) database of National Centre for Biological Information (NCBI), USA. The sequences producing significant alignments are shown along with its bits score and E values. It indicates the extent of homology amongst the sequence enquired (i.e. the 328 bp sequence from adil.flesh) and the sequences registered in *nr* database of NCBI. BLAST analysis revealed the highest homology of the sequence revealed from 'adil.flesh' with the sequence of *Panthera pardus* (gene bank registration number 'AY005809'), indicating the identity of adil.flesh as that of a leopard (*Panthera pardus*) origin. Figure 1b further illustrates the multiple alignments of the sequences obtained from reference animals (listed in Table 5) along with the sequence obtained from 'adil.flesh'. The sequences of 'adil.flesh' is similar to the sequences of 'gz1L' further confirming the identity of the source of confiscated remain 'adil.flesh' as that of a *Panthera pardus* origin.

Figure 1c illustrates the NJ-tree (Neighbor Joining tree) constructed using CLUSTAL X (1.8) from the sequences revealed from 'adil.flesh' and reference animals listed in Table 5. The animals belonging to similar species cluster together; however, the animals of different species group in different clusters. The confiscated material under investigation (i.e. 'adil.flesh') clusters with 'gz1L' (i.e. the known normal leopard '*Panthera pardus*') indicating the identity of the species of 'adil.flesh' as that of a *Panthera pardus* source.

Figure 2 shows the Agarose gel electrophorogram showing the PCR amplicons (472 bp) obtained from the reference animals of family felidae listed in Table 5, using universal primers 'mcb398 and 'mcb869'. Description of different lanes is as follows:

Lanes 1-21: The PCR profiles of the animals 1-21, respectively, listed in Table 5.

Lane 22: The PCR profiles of DNA isolated from confiscated skin of unknown animal origin 'i.e. adulterated'

Lane 23: Negative control (no DNA)

Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)

Figure 3. Shows PCR amplicons obtained from animals listed in Table 9. The primers used in PCR are 'AFF' and 'AFR'. The description of different lanes shown is as follows:

Lane 1-4: The PCR profiles of animals 1-4, respectively, listed in Table 9, showing amplicons of 354 bp.

Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)

Figure 4. Shows PCR amplicons obtained from animals listed in Table 12. This experiment demonstrates the universal nature of our primers among a vast range of animal species.

Description of different lanes shown is as follows:

Lanes 1-23: The PCR profiles of the animals 1-23, respectively, listed in Table 12. The PCR product of 472 bp is amplified universally from all the animal species analyzed.

Lane 24: Negative control (no DNA)

Lane M: Molecular weight marker (marker XIII, Boehringer mannheim)

Table 1. List of 221 animal species used for *In-silico* analysis to design the universal primers 'mcb398' and 'mcb869'. Table also demonstrate the 'P,S scores' of 'mcb398' and 'mcb869' for different templates. The descriptions of various symbols used in this table are as follows:

Symbol (#) refers to Number

Symbol (*) refers to the animal species which is either protected species (listed in Wildlife (Protection) Act , 1972 (Central Act NO 53 of 1972), or an endangered/rare animal species

Symbol (^{\$}P,S/F) refers to Probability of match and Stability of match of primer 'mcb398' with different templates (i.e. the cytochrome b gene from different species origin). A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Symbol (^ψP,S/R) refers to Probability of match and Stability of match of primer 'mcb869' with different templates. A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

the molecular basis of identification of individual animal species using our primers ‘mcb398’ and ‘mcb869’.

Table 7 (Tables 7a, 7b, 7c and 7d). The comparison of the molecular signatures of different animal species investigated along with ‘adil.flesh’, the confiscated skin of unknown animal origin. This table demonstrates the variable positions (i.e. the positions which are not marked with star (*) symbol in Table 6), amongst the 328 bp fragment revealed from the animals listed in Table 5. The dot (.) mark represents the presence of the similar nucleotide as listed in lane 1 i.e. the sequence from “adil.flesh” at that position. It demonstrates that the signatures of each species are unique and specific to its species. The molecular signatures of ‘adil.flesh’ are comparable (except for position 37 which has a transition from ‘T’ to ‘C’) to the molecular signature of ‘gz1L’ i.e. the known leopard ‘*Panthera pardus*’ source, indicating the identity of the source of confiscated skin ‘adil.flesh’ as that of a leopard ‘*Panthera pardus*’ source. The nucleotide variations (at the positions 153, 198, 223, 264, among the known leopards, (i.e. gz1L, gz2L, and gz3L, respectively)), give an idea about the geographical habitat of each animals. Various studies referring to molecular evolution of different animal species support this hypothesis⁷⁵; however, it could further be confirmed by taking the reference animals from different geographical areas and analyzing by our primers ‘mcb 398’ and ‘mcb869’. If we could generate the database of different haplotypes (i.e. habitat specific molecular signatures) of the animal species, it would also enable our primers to reveal the geographical location of the commitment of wildlife crime.

Table 8. Percent similarity matrix calculated by pair-wise comparisons of nucleotide sequences aligned (illustrated in Table 6). The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity (99.7% and 98.2%, with the lineages of animals ‘gz2L’ and ‘gz3L’, respectively) with the sequences obtained from known normal leopard source, indicating its identity as that of a leopard origin. The similarity matrix has been calculated using the software *PHYLIP* (3.5).

Table 9. Animals selected for validation of minimum P,S score for efficient amplification of cytochrome b gene of different origin by the primers ‘mcb398’ and ‘mcb869’. P,S score of primers ‘AFF’ and ‘AFR’ for these animals are shown.

Table 10. BLAST analysis of primers ‘mcb398’ in *nr* database of NCBI . It demonstrates that the 3’ end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer

Table 11. BLAST analysis of primers ‘mcb869’ in *nr* database of NCBI. It demonstrates that the 3’ end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer.

Table 12. Other animal belonging to distantly related animal species, investigated to confirm the universal nature of primers ‘mcb398’ and ‘mcb869’. Gel photograph showing the PCR amplicons from these animals are shown in Figure 4.

The mitochondrial cytochrome b gene has very widely been used in molecular taxonomic studies. It has immense capabilities to reveal different evolutionary lineages of animals in family, genus and species specific manner. It has also been used to classify the population of a particular species according to its demographic distributions⁷⁵. The vast database of cytochrome b sequences of different animal species has accumulated in public databases such as Genbank and NCBI¹⁻⁶⁵. We have explored these unique characteristics of cytochrome b gene to establish the identity of confiscated remains of any unknown animal by inventing a pair of novel primers, ‘mcb398’ and ‘mcb869’, that can amplify a small fragment (472 bp) of cytochrome b gene of wide range of animal species in universal manner. These primers work universally because its 3’ ends target within a highly conserved region.

The fragment of cytochrome b gene identified had all the features mentioned in columns 1, 2 and 3 listed under sub-heading ‘Objective of invention’. We identified this fragment by aligning the cytochrome b gene sequences (1140 bp) of 221 different animal species listed in Table 1. These sequences are publicly available in NCBI DNA databases. These sequences were aligned using the software *CLUSTAL X (1.8)*. As mentioned before, the 472 bp fragment of cytochrome b gene identified by us to have the features mentioned in columns 1, 2 and 3 listed under sub-heading ‘Objective of invention’ includes the nucleotides between 398 to 869 in *Antelope cervicapra* and *Felis catus*; however, 399 to 870 in *Homo sapiens sapiens* species. Except at few positions (marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, revealing the identity of the biological material belonging to that of an unknown animal origin by the procedure invented by us. As for identity of this fragment we are considering *Antelope cervicapra* as a representative species, and the sequence the above fragment of cytochrome b gene of *Antelope cervicapra* is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antilope cervicapra*

“taccatgaggacaaatatcttttgaggagcaacagtcacccaatctccttcagcaatccatacatcggtacaaacctagtaga
atgaatctgaggagggttctcagtagataaagcaaccctacccgattttcgcttccactttatcctcccatttatcattgcagccctta
ccatagtacacctactgtttctccacgaaacaggatccaacaacccccacaggaatctcatcagacgcagacaaaaattccattccacc
ctactacactatcaaagatatcctaggagctctactattaattttaaccctcatgcttctagtctattctcaccggacctgcttgagacc
cagacaactatacaccagcaaaccacttaatacacccccacatatcaagcccgaatgatacttctatttgcatacgcaatcctccga
tcaattcctaacaactaggagg

Table 2 presents the alignment of the above fragment of cytochrome b gene of 221 animal species. Each species in table 2 has been represented by a unique code, which is decoded in Table 1. We selected these species to represent the vast range of animal families of distant orders. Of 221 species, about 65 were the protected/endangered or rare species listed in Wildlife (Protection) Act , 1972 (Central Act NO 53 of 1972). These species are marked with symbol (*) in Table 1. The NCBI accession number refers to its registration number in NCBI database and the number in superscript represent the reference cited. Based on the aligned cytochrome b sequences of different 221 animal species the primers designed were as follow:

Primers name	Sequence (5'-3')
'mcb398'	“TACCATGAGGACAAATATCATTCTG”
'mcb869'	“CCTCCTAGTTTGTTAGGGATTGATCG”

Tables 2, 10 and 11, respectively, demonstrates that the 3' ends of the primers are highly conserved amongst all the animal species analyzed *in-silico* (In total 221 animal species listed in Table 1 and about 500 species listed in Tables 10 and 11, respectively) Also, the 5' end of the primers were selected within the conserved region of cytochrome b gene to improve the probability and stability of match of the primers to their target sequences (i.e. the above mentioned 472 bp fragment of cytochrome b gene). The primers were thoroughly checked for internal stabilities, loop or dimmer formation using different software viz., 'Amplify (1.2)', 'Primer3' (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>) as well as manually. . We assigned the P,S score (P=Probability of match, S=Stability of match) to the primers for each template using the software *Amplify (1.2)*. The higher scores of P and S ensure a good amplification if all other conditions standard (which are mentioned under 'Example 3') are optimum. The Highest score for 'mcb398' was 98,63 (i.e. the situation where the primer has perfect match with template); however, the highest P, S for 'mcb869' was recorded as 98, 68 for a complete match between the primer and template.

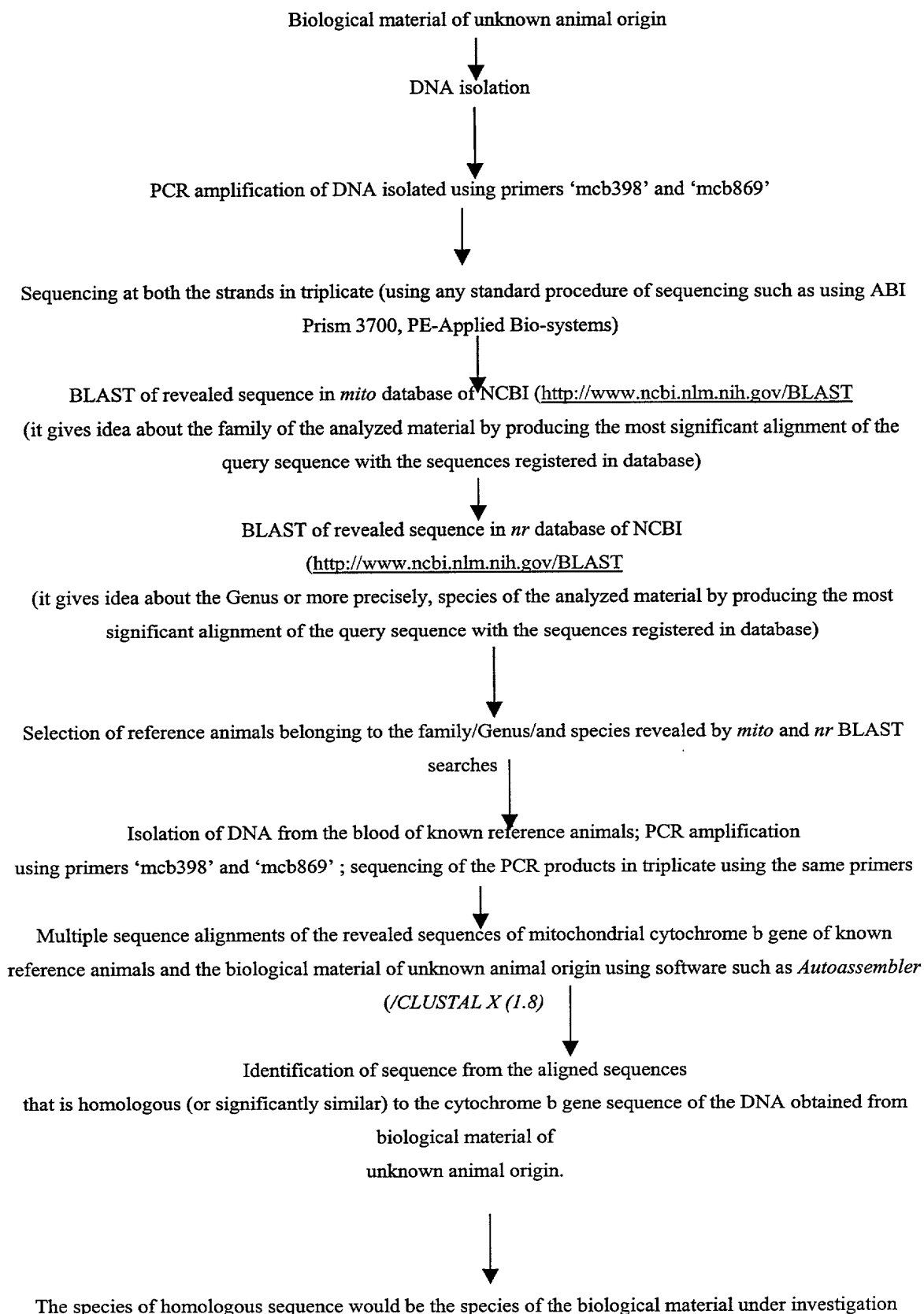
The lowest P,S score observed for ‘mcb398’ was 81,50 for species *Talpa europaea* whereas ‘mcb869’ had a high P, S score for this species (92, 57). The another species which have lowest P, S score for one of the two primers were *Eumeces egregius* and *Equus ainus*. *Eumeces egregius* had P, S score 86, 55 and 73,51 for ‘mcb398’ and ‘mcb869’, respectively; however, the P, S score of *Equus ainus* was calculated as 91,61 and 73, 51 for ‘mcb398’ and ‘mcb869’, respectively. All other animals had higher P, S scores then the above mentioned species. To ensure that these primers would work efficiently with the DNA template from the animals having the lowest P, S score for one of the primers, we designed an another experiment to validate the lower limits of one of the two primers sufficient for efficient amplification in PCR. We designed an another primer pair (AFF= 5’tagtagaatgaatctgaggagg3’ and AFR=5’atgcaaataggaagtatcattc3’.) having more mis-pairing at their annealing sites (but not at ends), therefore have less internal stability and lower P, S scores for its templates (listed in Table 9). The P,S scores of ‘AFF’ and ‘AFR’ were as calculated as low as 41 and 49 for *Platanista gangetica* and *Sus scrofa* These species were amplified efficiently using the primers ‘AFF’ and ‘AFR’ (results shown in Figure 3) (keeping all other conditions standard i.e. the conditions mentioned in ‘Example 3’). The lowest P,S scores (86, 55 and 73,51 for species *Eumeces egregius*) for our primers ‘mcb398’ and ‘mcb869’, respectively, were higher then the above range of combined P, S scores of ‘AFF’ and ‘AFR’ for species *Sus scrofa* (87, 52 and 87, 41), which was efficiently amplified by the primers ‘AFF’ and ‘AFR’. It gives an indication that the primers ‘mcb 398’ and ‘mcb 869’ would work with all the species including *Eumeces egregius* efficiently to give rise to the expected product in PCR. This experiment confirmed that the primers ‘mcb398’ and ‘mcb 869’ are capable of amplifying the cytochrome b fragment of most of the animal species in a universal manner.

For further confirmation of universal nature of our primers, we blasted the sequence of our primers against the *mito* and *nr* databases of NCBI using BLAST software. The results of these analyses are shown in Tables 10, and 11, respectively.

Finally, the universal nature of the primers was tested in our laboratory with some more animal species listed in Table 12. These primers amplified all the animal species efficiently, giving rise to the band of expected size (472 bp). The results are shown in Figure 4. This experiments substantiated the results of P,S analysis and other *in-silico* analyses to show that the primers ‘mcb398’ and ‘mcb 869’ are universal primers.

The flow chart of establishing identity of the species of biological material of unknown animal origin using primers ‘mcb398’ and ‘mcb869’

Flow chart of establishing identity of the species of biological material of unknown animal origin using primers ‘mcb398’ and ‘mcb869’



Examples

Example 1.

Example for identification of a fragment of cytochrome b gene fulfilling the requirements of columns 1, 2 and 3 mentioned under sub-heading 'Objectives of invention' of heading 'Brief summary of invention'

The cytochrome b molecule has very vastly been used in molecular taxonomic studies. Being a slow evolving gene, It has a tremendous information in its nucleotide sequences to distinguish the animals to their family, genus and species sources¹⁻⁶⁵. A vast database of the sequences of cytochrome b gene of different animal species has accumulated in the *nr* and *mito* databases of NCBI. We have explored these qualities of cytochrome b gene to establish the identity of confiscated remains of unknown animal origin to its family, genus and species sources. For this purpose, we have identified a fragment of cytochrome b gene which is highly polymorphic inter-specifically, however, it is monomorphic among the individual of same species, therefore it can group the individual of an unknown species with the known individuals of reference species to which it belongs. In order to amplify this fragment from DNA isolated from any unknown origin, it was necessary that it remain flanked with the highly conserved sequences amongst a vast range of animal families. To identify such a unique fragment within the cytochrome b gene, we aligned the sequences of 221 distantly related animal species (listed in Table 1) representing various families using software CLUSTAL X (1.8). These sequences were obtained from public database NCBI (<http://www.ncbi.nlm.nih.gov>). The aligned data was examined carefully for the conserved sites amongst all the species included in *in-silico* analysis. We identified a fragment (472 bp) of cytochrome b gene that was fulfilling all the requirements mentioned above and also under column 1, 2 and 3 of sub-heading 'Objectives of invention'.

As for the identity of this fragment we would like to mention that it includes the nucleotides between 398 to 869 in *Antelope cervicapra* and *Felis catus*; however, 399 to 870 in *Homo sapiens sapiens* species. Except at few positions marked as star (*) in Table 2, the nucleotide sequences of this fragment are highly variable amongst the animal species, giving rise to their unique molecular signature. These molecular signatures are characteristic of its species and form the basis of revealing the identity of the biological material of an

unknown animal origin by the procedure invented by us. Considering *Antilope cervicapra* as a representative species, the sequence of this fragment is mentioned herewith:

Mitochondrial cytochrome b gene sequence (398-869 bp) of *Antilope cervicapra*

“taccatgaggacaaatatcttttgaggagcaacagtcacccaatctccttcagcaatcccatacatcggtacaaacctagtaga
atgaatctgaggagggttctcagtagataaagcaacccttaccgatttttcgccttcactttatctctccatttatcattgcagcccta
ccatagtacacctactgttttccacgaaacaggatccaacaacccacaggaatctcatcagacgcagacaaaaattccattccaccc
ctactacactatcaaagatactctaggagctctactattaattttaaccctcatgcttctagtctatttccaccggacctgcttgagacc
cagacaactatacaccagaaacccacttaatacacccccacatatcaagccgaatgatacttcctattgcatacgcaatcctccga
tcaattcctaacaactaggagg”

Example 2:

Example for development of universal primers to amplify the fragment identified mentioned under ‘Example 1’

A pair of universal primer was designed which has the following features:

1. It targets the fragment identified (mentioned under ‘Example 1’) to amplify it in polymerase chain reaction (PCR).
2. Its 3’ and 5’ ends that are highly conserved (marked as star (*) in Table 2), amongst a vast range of animal species ensuring the amplification of the fragment mentioned above in a universal manner. The sequencing of the fragment amplified by these primes reveals the molecular signature of the species of analyzed material, which on comparison with the sequences of the known reference animals reveals the identity of the species of unknown biological material under investigation.
3. The t_m (melting temperature) of both primers was almost similar (about 58 degree centigrade) ensuring the significant annealing of both the primers to its template, therefore significant amplification of targeted region in PCR.
4. The internal stability and P, S, score of the primers were ensured higher while designing it. The possibilities of internal loop formation, dimmer formation etc were also excluded by selecting its sequence uniquely. This ensured that the primer would

be a good primer to be used in PCR for amplification of DNA from unknown animal origin.

5. The 3' end of the primers were ensured to have either 'G' or 'C' to increase the probability of strong bonding at its 3'ends, which is necessary for efficient amplification of DNA template in PCR. It also strengthens the universal nature of the primer.
6. The sequences of the primers were ensured to be unique so that it does not give rise to non-specific and spurious products in PCR leading to confusion. It improved the efficiency and quality of the technique invented by us.
7. These primers were named as 'mcb398' and 'mcb869' because of its property to amplify a region of mitochondrial cytochrome b gene between nucleotides 398 to 869 of *Antelope cervicapra*, a representative animal species for this invention. We took this animal species as representative species because the idea of developing such a novel primers came in the mind of inventors while they were working on the genome of this animal in Centre for Cellular and Molecular Biology, Hyderabad, India.
8. The sequences of the universal primers invented are as follows:

Primers name	Sequence (5'-3')
'mcb398'	"TACCATGAGGACAAATATCATTCTG"
'mcb869'	"CCTCCTAGTTTGTTAGGGATTGATCG"

Example 3:

Example for development of universal PCR conditions to ensure the amplification of a template of any unknown origin in PCR, hence strengthening the universal nature of the technique invented by us

The PCR conditions developed had the following unique features:

- 1 These were capable of amplifying the DNA template of any animal origin in an universal manner using the universal primers mentioned under 'Example 2'.
2. The conditions were selected to ensure the comparable annealing temperature for both the primers i.e. 'mcb398' and 'mcb869'.
3. The PCR conditions standardized herewith are universal; therefore, the possibility of PCR failure with a template of unknown origin due to non-standard conditions is

excluded. It ensures the universal nature of our technique to be used in wildlife forensics.

4. The universal conditions mentioned above are:

Amplification reactions should be carried out in 20 µl reaction volume containing approximately 20 ng of template DNA, 100µM each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl₂, 0.5 unit of *AmpliTaq* Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed should be: an initial denaturation at 95°C for 10 min, followed by 35 cycles each of denaturation at 95°C for 45 s, annealing at 51°C for 1 min, and extension at 72°C for 2 min. The extension step at 35th cycles should be held for 10 min.

Example 4:

Establishing the universal nature of our primer and experimental evidences to demonstrate the universal nature of primers:

The universal nature of the primers 'mcb398' and 'mcb 869' was ensured by the following measures:

(a) Selecting the primers from the aligned cytochrome b gene sequences of 221 animal of distantly related species:

The cytochrome b gene sequences (1140 bp) were aligned using software *CLUSTAL X* (1.8). The region of cytochrome b gene that was most conserved amongst 221 animal species was selected to design the primers.

(b) Selecting the 3' and 5' ends of the primers at the highly conserved positions of cytochrome b gene:

The 3' and 5' ends of the primers were ensured to anneal to a highly conserved position amongst 221 animal species representing a vast range of animal families. It was done to ensure an efficient amplification of all the species in PCR. These positions are shown with star (*) mark in Table 2.

(c) Ensuring either 'G' or 'C' at the 3' end of the primers:

It was ensured the primers to have either 'G' or 'C' at its 3' ends as these are the nucleotides that ensure the strong bonding at the 3' ends of the primers due to three hydrogen bonds while pairing with each other. The strong bonding at 3' ends helps the primers to anneal properly with its template resulting in significant amplification in PCR.

(d) Selecting the sequences of the primers to ensure a higher internal stability, higher P, S score, and no primer dimer and loop formation:

The sequences of the primers were selected to have a high P, S score for a vast range of animal species (Shown in Table 1). The care was taken to exclude the possibilities of loop or primer dimer formation that could reduce the efficiency of the primers in PCR.

(e) Selecting the sequence of the primers with a comparable melting temperature:

The sequences of the primers were selected to have a comparable melting temperature so that these could work together to amplify a DNA template in PCR at a similar annealing temperature. The melting temperature of both the primers was about 58 degree centigrade and the annealing temperature used in PCR is 51 degree centigrade.

Experimental evidences to demonstrate the universal nature of primers:

(1) Evidence from *In-silico* analysis :

(a) Selecting the primers within the most conserved region of mitochondrial cytochrome b gene

As mentioned above, the primers were designed to anneal within a highly conserved region of mitochondrial cytochrome b gene fragment of 472 bp. Table 2 presents the alignment of the above fragment of cytochrome b gene of 221 animal species representing a vast range of animal families. The conserved positions of nucleotide sequences are shown with star (*) mark in Table 2

Table 2 also demonstrates that the 3' ends of the primers are highly conserved amongst all the animal species analyzed *in-silico*. In the aligned sequences, the conserved nucleotides are marked with symbol (*). Also, the 5' end of the primers were selected within the conserved region of cytochrome b gene to improve the probability and stability of match of the primers to their target sequences (i.e. the above mentioned 472 bp fragment of cytochrome b gene). The primers were thoroughly checked for internal stabilities, loop or dimer formation using different software viz., '*Amplify (1.2)*', '*Primer3*' (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>) as well as manually.

(b) P, S, score analysis:

We assigned the P,S score (P=Probability of match, S=Stability of match) to the primers for each template using the software *Amplify (1.2)*. The higher scores of P and S ensure a good amplification if all other conditions standard (which are mentioned under 'Example 3') are optimum. The Highest score for 'mcb398' was 98,63 (i.e. the situation

where the primer has perfect match with template); however, the highest P, S for ‘mcb869’ was recorded as 98, 68 for a complete match between the primer and template. The lowest P,S score observed for ‘mcb398’ was 81,50 for species *Talpa europaea* whereas ‘mcb869’ had a high P, S score for this species (92, 57). The another species which have lowest P, S score for one of the two primers were *Eumeces egregius* and *Equus ainus*. *Eumeces egregius* had P, S score 86, 55 and 73,51 for ‘mcb398’ and ‘mcb869’, respectively; however, the P, S score of *Equus ainus* was calculated as 91,61 and 73, 51 for ‘mcb398’ and ‘mcb869’, respectively. All other animals had higher P, S scores then the above mentioned species. To ensure that these primers would work efficiently with the DNA template from the animals having the lowest P, S score for one of the primers, we designed an another experiment to validate the lower limits of one of the two primers sufficient for efficient amplification in PCR. We designed an another primer pair (AFF= 5’ctagtagaatgaatctgaggagg³’ and AFR= 5’tatgcaaataaggaagtatcattc³’) that have more mis-pairing at their annealing sites (but not at ends), therefore have less internal stability and lower P, S scores for its templates (listed in Table 9). The P,S scores of ‘AFF’ and ‘AFR’ were as calculated as low as 41 and 49 for *Platanista gangetica* and *Sus scrofa*. These species were amplified efficiently using the primers ‘AFF’ and ‘AFR’ (results shown in Figure 3) (keeping all other conditions standard i.e. the conditions mentioned in ‘Example 3’). The lowest P,S scores (86, 55 and 73,51 for species *Eumeces egregius*) for our primers ‘mcb398’ and ‘mcb869’, respectively, were higher then the above range of combined P, S scores of ‘AFF’ and ‘AFR’ for species *Sus scrofa* (87, 52 and 87, 41), which was efficiently amplified by the primers ‘AFF’ and ‘AFR’. It gives an indication that the primers ‘mcb 398’ and ‘mcb 869’ would work with all the species including *Eumeces egregius* efficiently to give rise to the expected product in PCR. This experiment confirmed that the primers ‘mcb398’ and ‘mcb 869’ are capable of amplifying the cytochrome b fragment of most of the animal species in a universal manner.

© BLAST analysis:

The sequences of primers ‘mcb398’ and ‘mcb869’ were blasted against mito and nr databases of NCBI to see its significant alignments with the sequences registered in GenBank. As expected, the most significant alignments of the sequences were found with the cytochrome b gene regions (within the 472 bp fragment mentioned in ‘Example 1’) of different animal species. This analysis also showed that the 3’ as well as 5’ ends of the primers were highly conserved amongst a vast range of animal species, confirming the universal nature of the primers (Tables 10 and 11, respectively)

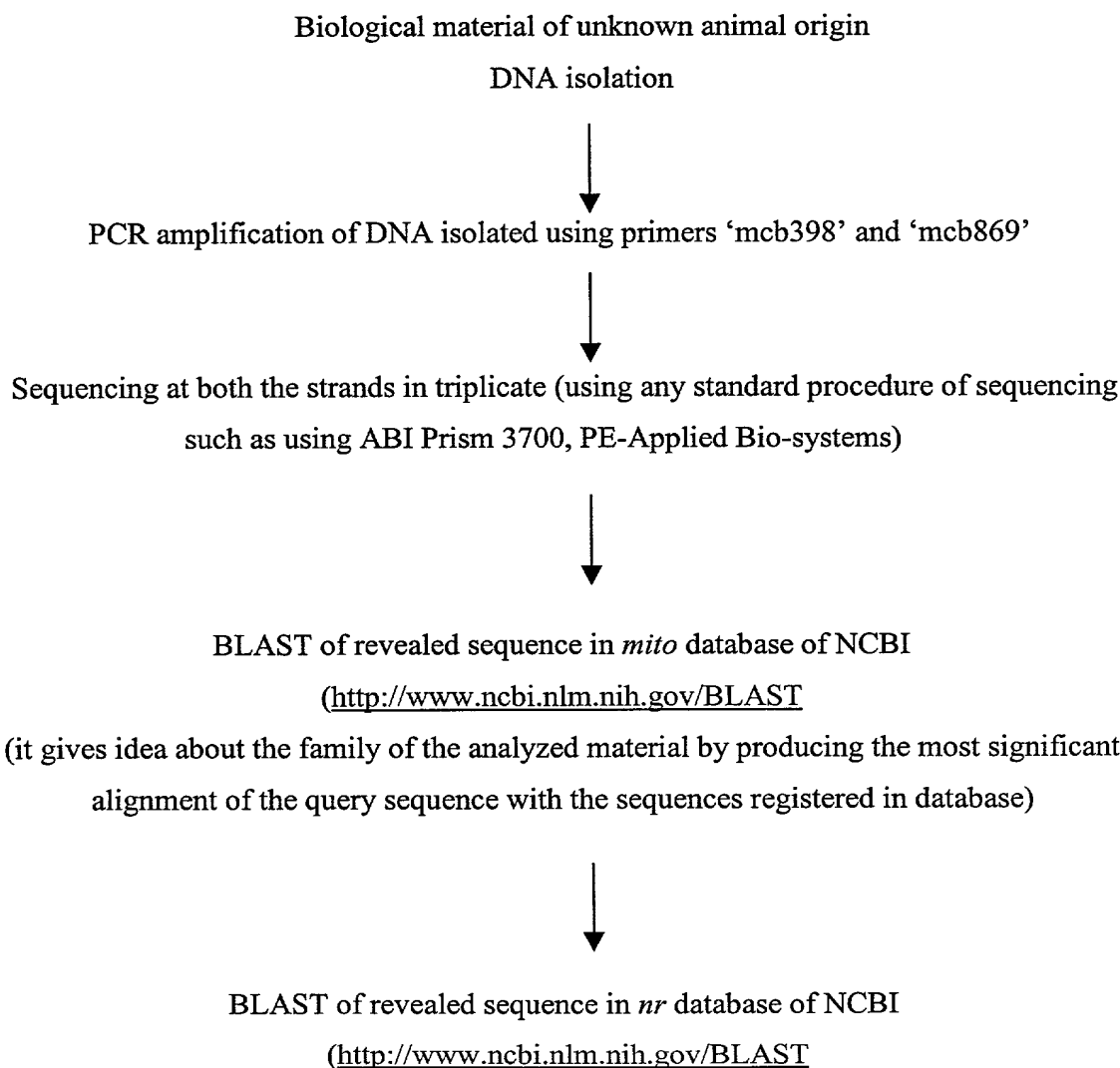
(2) Evidence from bench work/experiments done in laboratory conditions:

The DNA from different animals belonging to distantly related species (mentioned in Table 12) was isolated and subjected to PCR amplification using the primers invented by us i.e. the primers 'mcb398' and 'mcb869'. The PCR products amplified were resolved in agarose gel by electrophoresis and visualized under UV light. The PCR products of expected size (472bp) were obtained from all the animals confirming the universal nature of our primers. These results are shown in Figure 4.

Example 5:

Example to establish the identity of confiscated remains from unknown animal origin using the universal primers 'mcb398' and 'mcb869'.

The step-wise procedure to establish the identity of the biological material from an unknown animal source is mentioned below:



(it gives idea about the Genus or more precisely, species of the analyzed material by producing the most significant alignment of the query sequence with the sequences registered in database)



Selection of reference animals belonging to the family/Genus/and species revealed by *mito* and *nr* BLAST searches



Isolation of DNA from the blood of known reference animals;
PCR amplification using primers 'mcb398' and 'mcb869' ; sequencing of the PCR products in triplicate using the same primers



Multiple sequence alignments of the revealed sequences of mitochondrial cytochrome b gene of known reference animals and the biological material of unknown animal origin using software such as *Autoassembler/CLUSTAL X (1.8)*



Identification of sequence from the aligned sequences that is homologous (or significantly similar) to the cytochrome b gene sequence of the DNA obtained from biological material of unknown animal origin.



The species of homologous sequence would be the species of the biological material under investigation



Application of the above information for the objectives mentioned in columns 7-13 under sub-heading 'Objective of invention' of heading 'Summary of invention'

Example 6:

The actual execution of the technique invented

As a first application and to demonstrate the ease and utility of this method, we investigated a case of forensic identification submitted at our laboratory to seek scientific opinion on animal hunting evidence. In this case, we received the half burned remains of an unknown animal, confiscated by the crime investigation agencies. The DNA was isolated from the above material following standard methods⁷⁴ and subjected to PCR amplification using the primers mentioned above (viz., 'mcb398' and 'mcb869'). Amplification reactions were carried out in 20 µl reaction volume containing 20 ng of template DNA, 100µm each of dNTPs, 1.25 pmole of each primer, 1.5mM MgCl₂, 0.5 unit of AmpliTaq Gold (Perkin-Elmer-Cetus, USA) DNA polymerase and 1X PCR buffer (10mM Tris-HCl, pH 8.3, and 50mM KCl). The amplification profiles followed were: an initial denaturation at 95°C for 10 min, followed by 35 cycles each of denaturation at 95°C for 45 s, annealing at 51°C for 1 min, and extension at 72°C for 2 min. The extension step at 35th cycles was held for 10 min.

The PCR products obtained were sequenced in automated work station (ABI Prism 3700, PE-Biosystems) on both strands in triplicate and the sequence resolved (328 bp, shown in Figure 1a) was blasted against *mito* databases of NCBI using BLAST program⁷³. The most significant alignment (bits Value 365, E value e^{-101}) of this sequence was produced with the cytochrome b gene sequence of *Felis catus*, (Table 3) indicating that species of analyzed material belongs to family felidae. Further, the above sequence revealed from the confiscated remain was blasted against *nr* databases of NCBI using BLAST program. The most significant alignment (bits Value 603, E value e^{-170}) of this sequence was produced with the cytochrome b gene sequence of *Panthera pardus* (Table 4), indicating the identity of the analyzed material as that of a *Panthera pardus* source. Based on this information, we selected the reference animals listed in Table 5 representing different species and subspecies of felidae. The DNA isolated from reference animals was amplified and sequenced on both strands in triplicate using the primer pair mentioned above. Consensus sequences obtained were aligned using program *CLUSTAL X* (1.8) (Table 6). Sequence comparisons identified 113 variable sites in total amongst all animals analyzed (Table 7). Pair-wise comparisons of sequences were performed to find out the variation among different animals investigated. All the species investigated were differentiated by a their unique nucleotides sequences. The molecular signatures of different reference animals

were compared with the molecular signature of the confiscated skin 'adil.flesh'. Table 7 demonstrate that the maximum similarity of the adil.flesh with 'gz11' i.e. known Leopard (*Panthera pardus*) species, indicating the identity of the adil.flesh, the confiscated skin, as that of a *Panthera pardus* origin. We also calculated the similarity matrix showing the pair-wise similarity amongst the animal species under investigation using *PHYLIP* software. This matrix is shown in Table 8. It demonstrates that the animals belonging to different species had more variation; however, the animals of same species had maximum similarity among their cytochrome b sequences. The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity with the sequences obtained from known Leopard source (99.7%, and 98.2 with 'gz11' and 'gz21', respectively); establishing the identity of the source of confiscated material as that of a Normal leopard (*Panthera pardus*) species. The step-wise procedure involved in above analysis is illustrated in Figure 1a, 1b and 1c, respectively.

Thus, the primers invented by us can generate the molecular signature from any biological material of unknown animal origin, which actually is the characteristic of its family, genus and more precisely, the species. When these signatures are compared *in-silico* with the signatures already available in public databases (viz., GenBank, NCBI database etc) using *BLAST* software⁷³, it indicates identity of the family, genus or species of the analyzed material, which in turn is confirmed practically by comparing with the reference animals of the revealed family, genus or species, by including them in the further analysis by the primers 'mcb398' and 'mcb869'. Application of the information revealed could be in fulfilling the requirements of objectives mentioned in columns 7-13 under sub-heading 'Objective of invention' of heading 'Summary of invention'

The method of the invention can be used to establish the identity of confiscated animal parts and products is one of the key requirements of wildlife identification in forensics. It is needed to establish the crime with the criminal beyond a reasonable doubt to avoid the human violation of wildlife resources. Various morphological biochemical and molecular approaches have been given for this purpose; however, none of the current methods is universally applicable to detect the mutilated animal remains of unknown origin. We have identified a fragment on the mitochondrial cytochrome b gene, which has enormous information to differentiate among various animal species back to the family, genus and species sources. We have also found that this fragment is flanked by the highly conserved sequences amongst a vast range of animal species. We invented a pair of universal primer

Table 1. List of 221 animal species used for *In-silico* analysis to design the universal primers ‘mcb398’ and ‘mcb869’. Table also demonstrate the ‘P,S scores’ of ‘mcb398’ and ‘mcb869’ for different templates. The descriptions of various symbols used in this table are as follows:

Symbol (#) refers to Number

Symbol (*) refers to the animal species which is either protected species (listed in Wildlife (Protection) Act , 1972 (Central Act NO 53 of 1972), or an endangered/rare animal species

Symbol (^{\$}P,S/F) refers to Probability of match and Stability of match of primer ‘mcb398’ with different templates (i.e. the cytochrome b gene from different species origin). A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Symbol (^ψP,S/R) refers to Probability of match and Stability of match of primer ‘mcb869’ with different templates. A higher P,S score refers to the higher probabilities of significant amplification of specific template by the primer. It is calculated by *Amplify (1.2)* software.

Table 1. The animal species included in the study for *in-silico* analysis

SN. Code	Name	NCBI accession #	[§] P,S/F	[¶] P,S/R
1 aep.mel	<i>Aepyceros melampus</i>	AF036289 ¹	97, 60	94, 62
2 ore.ore	<i>Oreotragus oreotragus</i>	AF036288 ¹	88, 52	94, 62
3 add.nas	<i>Addax nasomaculatus</i>	AF034722 ²	97, 60	95, 66
4 ory.dam	<i>Oryx damah</i>	AJ222685 ¹	90, 58	95, 66
5 hip.equ	<i>Hippotragus equinus</i>	AF022060 ³	98, 63	85, 55
6 alc.bus	<i>Alcelaphus buselaphus</i>	AJ222681 ¹	97, 60	98, 68
7 sig.lic	<i>Sigmoceros lichtensteinii</i>	AF034967 ⁴	97, 60	98, 68
8 bea.hun	<i>Beatragus hunteri</i>	AF034968 ⁴	97, 60	94, 62
9 dam.lun	<i>Damaliscus lunatus</i>	AF016635 ³	97, 60	77, 55
10 con.tau	<i>Connochaetes taurinus</i>	AF016638 ³	82, 56	93, 62
11 bis.bon	<i>Bison bonasus</i>	Y15005 ⁵	90, 58	87, 63
12 bos.gru	<i>Bos grunniens</i> *	AF091631 ⁶	90, 58	94, 62
13 bos.tra	<i>Bos tragocamelus</i> *	AJ222679 ¹	90, 58	95, 66
14 buba.bub	<i>Bubalus bubalis</i> *	D34637 ⁷	97, 60	93, 64
15 bub.min	<i>Bubalus mindorensis</i>	D82895 ⁸	97, 60	87, 62
16 tra.ang	<i>Tragelaphus angasii</i>	AF091633 ⁶	97, 60	87, 63
17 tra.eur	<i>Tragelaphus eurycerus</i>	AF036276 ¹	90, 58	97, 64
18 nem.cau	<i>Nemorhaedus caudatus</i> *	U17861 ⁹	95, 61	93, 59
19 pse.nay	<i>Pseudois nayaaur</i>	AF034732 ²	89, 55	89, 59
20 amm.ler	<i>Ammotragus lervia</i>	AF034731 ²	94, 58	97, 63
21 cap.fal	<i>Capra falconeri</i> *	D84202 ¹⁰	98, 63	95, 66
22 cap.ibe	<i>Capra ibex</i> *	AF034735 ²	98, 63	89, 58
23 hem.jem	<i>Hemitragus jemlahicus</i> *	AF034733 ²	95, 61	90, 61
24 rup.pyr	<i>Rupicapra pyrenaica</i>	AF034726 ²	95, 61	89, 59
25 rup.rup	<i>Rupicapra rupicapra</i>	AF034725 ²	95, 61	94, 64
26 pan.hod	<i>Pantholops hodgsoni</i>	AF034724 ²	98, 63	95, 66
27 bud.tax.tax	<i>Budorcas taxicolor taxicolor</i> *	U17868 ⁹	90, 58	95, 66
28 ovi.amm	<i>Ovis ammon</i> *	AF034727 ²	98, 63	97, 64
29 ovi.vig	<i>Ovis vignei</i> *	AF034729 ²	98, 63	97, 64
30 cap.cri	<i>Capcornis crispus</i> *	AJ304502 ¹¹	98, 63	94, 63
31 ovi.mos	<i>Ovibos moschatus</i>	U17862 ⁹	98, 63	92, 61
32 ore.ame	<i>Oreamnos americanus</i>	AF190632 ¹²	98, 63	94, 62
33 cep.dor	<i>Cephalophus dorsalis</i>	AF091634 ⁶	97, 58	90, 61
34 cep.max	<i>Cephalophus maxwellii</i>	AF096629 ¹³	97, 60	88, 53
35 alc.alc	<i>Alces alces</i>	AJ000026 ¹⁴	95, 61	93, 59
36 hyd.ine	<i>Hydropotes inermis</i>	AJ000028 ¹⁴	97, 60	90, 63
37 mun.mun	<i>Muntiacus muntjak</i> *	AF042718 ¹⁵	90, 58	93, 64
38 cer.ele.kan	<i>Cervus elaphus kansuensis</i> *	AB021098 ¹⁶	98, 63	82, 59
39 cer.ele.xan	<i>Cervus elaphus xanthopygus</i> *	AB021097 ¹⁶	98, 63	82, 59
40 cer.ele.can	<i>Cervus elaphus canadensis</i> *	AB021096 ¹⁶	98, 63	90, 61
41 cer.nip.ce	<i>Cervus nippon centralis</i>	AB021094 ¹⁶	98, 63	90, 61
42 cer.nip.ye	<i>Cervus nippon yesoensis</i>	AB021095 ¹⁶	98, 63	90, 61
43 cer.nip.ke	<i>Cervus nippon keramae</i>	AB021091 ¹⁶	98, 63	90, 61
44 cer.nip.pu	<i>Cervus nippon pulchellus</i>	AB021090 ¹⁶	98, 63	90, 61
45 cer.nip.ni	<i>Cervus nippon nippon</i>	AB021093 ¹⁶	98, 63	90, 61
46 cer.el.sc	<i>Cervus elaphus scoticus</i>	AB021099 ¹⁶	98, 63	90, 61

47	cer.dam	<i>Cervus dama</i>	AJ000022 ¹⁴	98, 63	88, 53
48	ran.tar	<i>Rangifer tarandus</i>	AJ000029 ¹⁴	98, 63	89, 57
49	mos.fus	<i>Moschus fuscus</i> *	AF026888 ¹⁷	90, 59	90, 61
50	mos.leu	<i>Moschus leucogaster</i> *	AF026889 ¹⁷	90, 59	90, 61
51	mos.chr	<i>Moschus chrysogaster</i> *	AF026887 ¹⁷	90, 59	90, 61
52	mos.ber	<i>Moschus berezovskii</i> *	AF026886 ¹⁷	90, 59	90, 61
53	mos.mos	<i>Moschus moschiferus</i> *	AF026883 ¹⁷	90, 59	92, 61
54	kob.ell	<i>Kobus ellipsiprymnus</i>	AF022059 ³	91, 61	95, 66
55	kob.meg	<i>Kobus megaceros</i>	AJ222686 ¹	91, 61	83, 56
56	red.aru	<i>Redunca arundinum</i>	AF096628 ¹³	91, 61	94, 62
57	red.ful	<i>Redunca fulvorufula</i>	AF036284 ¹	89, 57	94, 62
58	neo.mos	<i>Neotragus moschatus</i>	AJ222683 ¹	89, 57	94, 62
59	pel.cap	<i>Pelea capreolus</i>	AF022055 ³	91, 61	90, 61
60	ant.cer	<i>Antilope cervicapra</i> *	AF022058 ³	82, 56	93, 64
61	sai.tat	<i>Saiga tatarica</i>	AF064487 ¹⁸	91, 61	92, 61
62	gaz.dam	<i>Gazella dama</i>	AF025954 ³	91, 61	92, 61
63	our.our	<i>Ourebia ourebi</i>	AF036288 ¹	82, 56	82, 59
64	gaz.gaz	<i>Gazela gazella</i> *	AJ222682 ¹	91, 61	89, 57
65	rap.mel	<i>Raphicerus melanotis</i>	AF022053 ³	81, 54	80, 50
66	mad.kir	<i>Madoqua kirkii</i>	AF022070 ³	90, 58	97, 65
67	ant.ame	<i>Antilocapra americana</i>	AF091629 ⁶	98, 63	98, 68
68	tra.jav	<i>Tragulus javanicus</i> *	D32189 ¹⁹	86, 57	86, 59
69	tra.nap	<i>Tragulus napu</i> *	X56288 ²⁰	81, 52	93, 58
70	bal.acu	<i>Balaenoptera acutorostrata</i>	X75753 ²¹	89, 56	97, 61
71	bal.bon	<i>Balaenoptera bonaerensis</i>	X75581 ²¹	89, 56	93, 59
72	bal.bor	<i>Balaenoptera borealis</i> *	X75582 ²¹	89, 56	93, 59
73	bal.edi	<i>Balaenoptera edeni</i>	X75583 ²¹	89, 56	88, 54
74	esc.rob	<i>Eschrichtius robustus</i> *	X75585 ²¹	97, 61	86, 57
75	bal.mus	<i>Balaenoptera musculus</i> *	NC_001601 ²²	97, 57	93, 59
76	meg.nov	<i>Megaptera novaeangliae</i> *	X75584 ²¹	97, 61	94, 63
77	bal.phy	<i>Balaenoptera physalus</i> *	NC_001321 ²³	97, 57	94, 63
78	cap.mar	<i>Caperea marginata</i>	X75586 ²¹	93, 55	91, 53
79	cep.com	<i>Cephalorhynchus commersonii</i>	AF084073 ²⁴	85, 51	88, 55
80	cep.eut	<i>Cephalorhynchus eutropia</i> *	AF084072 ²⁴	85, 51	92, 59
81	lag.obl	<i>Lagenorhynchus obliquidens</i>	AF084067 ²⁴	94, 59	92, 59
82	cep.heu	<i>Cephalorhynchus heavisidii</i>	AF084070 ²⁴	89, 56	97, 63
83	cep.hec	<i>cephalorhynchus hectori</i> *	AF084071 ²⁴	89, 56	92, 59
84	lag.aus	<i>Lagenorhynchus australis</i>	AF084069 ²⁴	86, 54	92, 59
85	lag.cru	<i>Lagenorhynchus cruciger</i>	AF084068 ²⁴	86, 54	92, 59
86	lag.obs	<i>Lagenorhynchus obscurus</i>	AF084066 ²⁴	86, 54	92, 59
87	lis.bor	<i>Lissodelphis borealis</i>	AF084064 ²⁴	85, 51	92, 59
88	lis.per	<i>Lissodelphis peronii</i>	AF084065 ²⁴	86, 54	92, 59
89	glo.mac	<i>Globicephala macrorhynchus</i>	AF084055 ²⁴	94, 59	88, 55
90	glo.mel	<i>Globicephala melas</i>	AF084056 ²⁴	94, 59	88, 55
91	fer.att	<i>Feresa attenuata</i> *	AF084052 ²⁴	94, 59	92, 59
92	pep.ele	<i>Peponocephala electra</i> *	AF084053 ²⁴	94, 59	88, 55
93	gra.gri	<i>Grampus griseus</i>	AF084059 ²⁴	97, 61	89, 59
94	pse.cra	<i>Pseudorca crassidens</i> *	AF084057 ²⁴	94, 59	92, 59
95	lag.acu	<i>Lagenorhynchus acutus</i>	AF084075 ²⁴	98, 63	89, 59
96	orci.bre	<i>Orcinus orca</i>	AF084061 ²⁴	86, 57	82, 52

97	orca.bre	<i>Orcaella brevirostris</i>	AF084063 ²⁴	86, 57	91, 54
98	del.cap	<i>Delphinus capensis</i>	AF084087 ²⁴	96, 54	97, 63
99	del.tro	<i>Delphinus tropicalis</i>	AF084088 ²⁴	97, 57	97, 63
100	del.del	<i>Delphinus delphis</i>	AF084085 ²⁴	97, 57	97, 63
101	sten.cly	<i>Stenella clymene</i>	AF084083 ²⁴	97, 57	97, 63
102	sten.coe	<i>Stenella coeruleoalba</i>	AF084082 ²⁴	97, 57	97, 66
103	tur.adu	<i>Tursiops aduncus</i>	AF084092 ²⁴	97, 57	97, 63
104	sten.fro	<i>Stenella frontalis</i>	AF084090 ²⁴	97, 57	97, 63
105	saus.chi	<i>Sousa chinensis</i>	AF084080 ²⁴	97, 57	88, 59
106	sten.lon	<i>Stenella longirostris</i>	AF084103 ²⁴	97, 61	97, 63
107	turs.tru	<i>Tursiops truncatus</i>	AF084095 ²⁴	97, 57	96, 59
108	lage.alb	<i>Lagenorhynchus alborostris</i>	AF084074 ²⁴	97, 61	97, 66
109	sten.bre	<i>Steno bredanensis</i>	AF084077 ²⁴	97, 61	94, 64
110	sota.flu	<i>Sotalia fluviatilis</i>	AF304067 ²⁵	97, 61	97, 63
111	del.leu	<i>Delphinapterus leucas</i>	U72037 ²⁶	97, 61	95, 66
112	mono.mon	<i>Monodon monoceros</i>	U72038 ²⁶	97, 61	95, 66
113	plat.gan	<i>Platanista gangetica*</i>	AF304070 ²⁵	97, 61	86, 59
114	plat.min	<i>Platanista minor*</i>	X92543 ²⁷	97, 61	86, 59
115	kogi.bre	<i>Kogia breviceps</i>	U72040 ²⁶	97, 59	90, 63
116	kogi.sim	<i>Kogia simus</i>	AF304072 ²⁸	96, 55	92, 63
117	phys.cat	<i>Physeter catodon</i>	AF304073 ²⁵	97, 57	80, 58
118	lipo.vex	<i>Lipotes vexillifer*</i>	AF304071 ²⁵	89, 56	88, 53
119	phoc.sin	<i>phocoena sinus</i>	AF084051 ²⁴	87, 49	92, 62
120	bera.bai	<i>Berardius bairdii</i>	X92541 ²⁷	96, 55	90, 59
121	ziph.car	<i>Ziphius cavirostris</i>	X92540 ²⁷	97, 61	89, 57
122	meso.eur	<i>Mesoplodon europaeus</i>	X92537 ²⁷	97, 57	90, 61
123	meso.bid	<i>Mesoplodon bidens</i>	X92538 ²⁷	97, 61	92, 61
124	meso.den	<i>Mesoplodon densirostris</i>	X92536 ²⁷	91, 61	94, 63
125	hype.amp	<i>Hyperoodon ampullatus*</i>	X92539 ²⁷	97, 61	90, 65
126	meso.per	<i>Mesoplodon peruvianus</i>	AF304074 ²⁸	97, 61	86, 58
127	pont.bla	<i>Pontoporia blainvillei</i>	AF304069 ²⁵	92, 59	88, 55
128	hipp.amp	<i>Hippopotamus amphibius</i>	Y08813 ²⁹	92, 58	95, 66
129	hex.lib	<i>Hexaprotodon liberiensis</i>	Y08814 ²⁹	98, 63	97, 66
130	rhin.son	<i>Rhinoceros sondaicus*</i>	AJ245725 ³⁰	90, 59	87, 61
131	cera	<i>Ceratotherium simum</i>	NC_001808 ³²	90, 59	90, 63
132	dic.sum	<i>Dicerorhinus sumatrensis</i>	AJ245723 ³⁰	90, 59	86, 57
133	equu	<i>Equus asinus</i>	NC_001788 ³¹	91, 61	73, 51
134	baby.bab	<i>Babyrousa babyrussa</i>	Z50106 ³³	89, 56	85, 56
135	phac.afr	<i>Phacochoerus africanus</i>	Z50090 ³³	90, 59	87, 54
136	sus.scr.ew	<i>Sus scrofa haplotype EWB3*</i>	AF136549 ³⁴	97, 57	83, 54
137	sus.bar	<i>Sus barbatus</i>	Z50107 ³³	97, 57	85, 55
138	lama.gla	<i>Lama glama</i>	U06429 ³⁵	89, 55	85, 53
139	lama.gua	<i>lama guanicoe</i>	Y08812 ²⁹	88, 54	86, 57
140	vic.vic	<i>Vicugna vicugna</i>	U06430 ³⁵	89, 55	85, 53
141	cam.bac	<i>Camelus bactrianus</i>	U06427 ³⁵	94, 58	86, 58
142	arc.for	<i>Arctocephalus forsteri</i>	X82293 ³⁶	97, 60	87, 64
143	arc.gaz	<i>Arctocephalus gazella</i>	X82292 ³⁶	94, 58	87, 64
144	eum.jub	<i>Eumetopias jubatus</i>	X82311 ³⁶	97, 57	86, 57
145	zal.cal	<i>Zalophus californianus</i>	X82310 ³⁶	89, 55	86, 57
146	odo.ros	<i>Odobenus rosmarus</i>	X82299 ³⁶	91, 61	81, 52

147	pho.vit	<i>Phoca vitulina</i>	X82306 ³⁶	90, 58	87, 64
148	pho.fascia	<i>Phoca fasciata</i>	X82302 ³⁶	98, 63	95, 66
149	pho.gro	<i>Phoca groenlandica</i>	X82303 ³⁶	92, 59	90, 61
150	cys.cri	<i>Cystophora cristata</i>	X82294 ³⁶	89, 56	87, 64
151	hyd.lep	<i>Hydrurga leptonyx</i>	X82297 ³⁶	89, 55	82, 54
152	lep.wed	<i>Leptonychotes weddelli</i>	X72005 ³⁷	98, 63	91, 66
153	mir.leo	<i>Mirounga leonina</i>	X82298 ³⁶	89, 55	82, 59
154	eri.bar	<i>Erignathus barbatus</i>	X82295 ³⁶	89, 56	87, 63
155	mon.sch	<i>Monachus schauinslandi</i>	X72209 ³⁷	91, 61	87, 60
156	hela.mal	<i>Helarctos malayanus</i> *	U18899 ³⁸	84, 54	90, 63
157	sel.thi	<i>Selenarctos thibetanus</i> *	AB020910 ³⁹	89, 57	87, 64
158	ail.ful	<i>Ailurus fulgens</i> *	X94919 ⁴⁰	93, 55	87, 64
159	fel	<i>Felis catus</i>	NC_001700 ⁴¹	85, 56	90, 63
160	can	<i>Canis familiaris</i>	NC_002008 ⁴²	98, 58	84, 54
161	tal	<i>Talpa europaea</i>	NC_002391 ⁴³	81, 50	92, 57
162	gla.sab	<i>Glaucomys sabrinus</i>	AF011738 ⁴⁴	90, 59	82, 54
163	gla.vol	<i>Glaucomys volans</i>	AB030261 ⁴⁵	90, 59	87, 60
164	hyl.pha	<i>Hylopates phayrei</i> *	AB030259 ⁴⁵	91, 61	81, 50
165	pet.set	<i>Petinomys setosus</i> *	AB030260 ⁴⁵	91, 61	81, 50
166	bel.pea	<i>Belomys pearsonii</i> *	AB030262 ⁴⁵	91, 61	87, 64
167	pte.mom	<i>Pteromys momonga</i> *	AB030263 ⁴⁵	97, 61	90, 63
168	gala.demi	<i>Galagoides demidoff</i>	AF271411 ⁴⁶	97, 58	87, 64
169	pero.pot	<i>Perodicticus potto</i>	AF271413 ⁴⁶	97, 60	87, 63
170	gala.mat	<i>Galago matschiei</i>	AF271409 ⁴⁶	97, 60	90, 61
171	gala.moh	<i>Galago moholi</i>	AF271410 ⁴⁶	97, 57	95, 66
172	oto.gar	<i>Otolemur garnettii</i>	AF271412 ⁴⁶	92, 58	87, 60
173	lor.tar	<i>Loris tardigradus</i> *	U53581 ⁴⁷	97, 60	93, 59
174	nyc.cou	<i>Nycticebus coucang</i> *	U53580 ⁴⁷	97, 60	95, 66
175	mus	<i>Mus musculus</i>	NC_001569 ⁴⁸	97, 60	86, 59
176	gorr	<i>Gorilla gorilla</i>	NC_001645 ⁴⁹	89, 57	80, 58
177	homo	<i>Homo sapiens sapiens</i>	NC_001807 ⁵⁰	96, 55	84, 64
178	dug.dug	<i>Dugong dugong</i> *	U07564 ⁵¹	97, 60	89, 59
179	ele.max	<i>Elephas maximus</i> *	AB002412 ⁵²	97, 60	76, 57
180	afr.con	<i>Afropavo congensis</i>	AF013760 ⁵³	97, 58	87, 63
181	pavo.mut	<i>Pavo muticus</i> *	AF013763 ⁵³	97, 57	87, 63
182	tra.bly	<i>Tragopan blythii</i> *	AF200722 ⁵⁴	89, 55	85, 57
183	tra.sat	<i>Tragopan satyra</i> *	AF229837 ⁵⁴	89, 55	86, 61
184	tra.cob	<i>Tragopan caboti</i>	AF200723 ⁵⁴	89, 55	86, 61
185	tra.tem	<i>Tragopan temminckii</i> *	AF028802 ⁵⁵	89, 55	81, 56
186	arg.arg	<i>Argusianus argus</i>	AF013761 ⁵³	89, 55	87, 63
187	cat.wal	<i>Catreus wallichi</i> *	AF028792 ⁵³	88, 54	85, 57
188	cro.cro	<i>Crossoptilon crossoptilon</i> *	AF028794 ⁵³	89, 55	85, 57
189	sym.ree	<i>Syrnaticus reevesi</i> *	AF028801 ⁵³	89, 55	85, 57
190	bam.tho	<i>Bambusicola thoracica</i> *	AF028790 ⁵³	80, 48	94, 64
191	fra.fra	<i>Francolinus francolinus</i>	AF013762 ⁵³	97, 58	86, 61
192	ith.cru	<i>Ithaginis cruentus</i> *	AF068193 ⁵³	98, 63	85, 57
193	ant.par	<i>Anthropoides paradisea</i>	U27557 ⁵⁶	85, 56	82, 58
194	ant.vir	<i>Anthropoides virgo</i>	U27545 ⁵⁶	84, 54	82, 52
195	gru.ant.an	<i>Grus antigone antigone</i>	U11060 ⁵⁷	90, 58	87, 63
196	gru.ant.gi	<i>Grus antigone gillae</i>	U11064 ⁵⁷	90, 58	87, 63

197	gru.any.sh	<i>Grus antigone sharpei</i>	U11061 ⁵⁷	90, 58	87, 63
198	gru.leu	<i>Grus leucogeranus</i> *	U27549 ⁵⁶	90, 58	87, 63
199	gru.can.pr	<i>Grus canadensis pratensis</i>	U27553 ⁵⁶	97, 60	87, 63
200	gru.can.ro	<i>Grus canadensis rowani</i>	U27552 ⁵⁶	97, 60	87, 63
201	gru.can.ta	<i>Grus canadensis tabida</i>	U27551 ⁵⁶	98, 63	87, 63
202	gru.can.ca	<i>Grus canadensis canadensis</i>	U27554 ⁵⁶	97, 61	87, 63
203	gru.ame	<i>Grus americana</i>	U27555 ⁵⁶	90, 58	87, 63
204	gru.gru	<i>Grus grus</i>	U27546 ⁵⁶	89, 54	87, 63
205	gru.mon	<i>Grus monacha</i> *	U27548 ⁵⁶	90, 58	87, 63
206	gru.nig	<i>Grus nigricollis</i> *	U27547 ⁵⁶	90, 58	87, 63
207	gru.jap	<i>Grus japonensis</i>	U27550 ⁵⁶	81, 54	87, 63
208	cic.boy	<i>Ciconia boyciana</i> *	NC_002196 ⁵⁸	94, 58	79, 60
209	rhe.ame	<i>Rhea americana</i>	AF090339 ⁵⁹	93, 63	79, 60
210	ant.alb	<i>Anthracoseros albirostris</i> *	U89190 ⁶⁰	97, 61	86, 59
211	fal.fam	<i>Falco femoralis</i>	U83310 ⁶¹	97, 61	86, 60
212	fal.ver	<i>Falco verpertinus</i>	U83311 ⁶¹	97, 61	85, 57
213	fal.par	<i>Falco peregrinus</i> *	U83307 ⁶¹	97, 61	84, 52
214	fal.spa	<i>Falco sparverius</i>	U83306 ⁶¹	92, 59	80, 51
215	ayt.ame	<i>Aythya americana</i>	NC_000877 ⁶²	98, 63	94, 62
216	smi.sha	<i>Smithornis sharpei</i>	NC_000879 ⁵⁹	97, 58	90, 61
217	vid.cha	<i>Vidua chalybeata</i>	NC_000880 ⁵⁹	97, 60	87, 64
218	chry.pic	<i>Chrysemys picta</i>	NC_002073 ⁶³	89, 56	86, 57
219	emy.orb.ku	<i>Emys orbicularis</i>	AJ131425 ⁶⁴	90, 59	94, 63
220	che.mud	<i>Chelonia mydas</i> *	AB012104 ⁶⁵	90, 58	94, 63
221	eum.egr	<i>Eumeces egregius</i>	AB016606 ⁶⁵	86, 55	73, 51

Table 2. Multiple sequence alignment of 472 bp fragment of mitochondrial cytochrome b gene (identified by inventors to fulfill the requirements of column 1, 2 and 3 mention under sub-heading ‘Objectives of invention’) of 221 animal species listed in Table 1. Alignments also show the binding sites for universal primers ‘mcb398’ and ‘mcb869’. The symbol (*) refers to the nucleotide bases which are conserved amongst 221 animal species listed in Table 1). The alignments have been done using software *CLUSTAL X (1.8)*. The nucleotide positions that are unmarked are variable amongst 221 animal species analyzed. These variable sites together constitute the molecular signature of an individual species, giving rise to molecular basis of species identification by our primers.

Table 2. Multiple sequence alignment of 472 bp fregment of mitochondrial cytochrome b gene of 221 animal species

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PRIMER 'mcb398'      TACCATGAGGACAAATATCATTCTG
* * * * *
** **
aep.mel
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATTACAAATCTCCTCTCAGCAA 60
ore.ore
TTCCGTGAGGACAAATATCATTTTGAGGGGCTACAGTCATTACTAATCTCCTCTCAGCAA 60
add.nas
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
ory.dam
TACCATGAGGACAAATATCATTTTGAGGGGCAACAGTTATCACTAACCTTCTCTCAGCAA 60
hip.equ
TACCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
alc.bus
TGCCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAATCTCCTCTCAGCAA 60
sig.lic
TGCCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAATCTCCTCTCAGCAA 60
bea.hun
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
dam.lun
TGCCATGAGGACAAATATCATTCTGAGGAGCAACAGTCATCACTAACCTCCTCTCAGCAA 60
con.tau
TACCATGAGGACAAATATCCTTTTGAGGAGCAACAGTCATCACCAACCTCCTCTCAGCAA 60
amm.ler
TGCCATGAGGACAGATATCATTCTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
pse.nay
TGCCATGAGGACAAATATCATTTTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
cap.ibe
TACCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACTAACCTTCTCTCAGCAA 60
hem.jem
TACCATGAGGACAGATATCATTCTGAGGGGCAACAGTCATCACCAACCTTCTCTCAGCAA 60
cap.fal
TACCATGAGGACAAATATCATTCTGAGGGGCAACAGTCATCACCAATCTCCTCTCAGCAA 60
rup.pyr
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rup.rup
TACCATGAGGACAGATATCATTCTGGGGAGCAACAGTTATTACCAACCTCCTCTCAGCGA 60
nem.cau
TACCATGAGGACAGATATCATTCTGAGGGGCAACAGTTATTACCAATCTTCTCTCAGCAA 60
bud.tax.tax
TACCATGAGGACAAATATCATTTTGAGGAGCAACAGTCATTACCAACCTCCTCTCAGCAA 60
pan.hod
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ovi.amm
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ovi.vig
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cap.cri
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ovi.mos
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ore.ame
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cep.dor
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cep.max
TCCCATGAGGACAAATATCATTCTGAGGAGCCACAGTCATTACCAACCTCCTCTCAGCAA 60

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 bos.tra
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 bubu.bub
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 tra.eur
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 kob.meg
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 red.aru
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 red.full
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 gaz.dam
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 our.our
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 ant.cer
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 rap.mel
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 hyd.ine
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 cer.nip.pul

glo.mac
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orci.bre
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orca.bre
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del.cap
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saus.chi
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Table 3. Results of the blast analysis of the sequence revealed from ‘adil.flesh’ in ‘mito’ database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece ‘adil.flesh’ with *felis catus* cytochrome b gene sequence (genbank registration number NC_001700.1, bits score 365, E value, e-101) registered in NCBI database (bits score 365 and E value e-101). It gives an indication that the species of analyzed material belongs to family felidae. It also fulfills the requirements of column 6 mention above under sub-heading ‘Objectives of invention’.

Downloaded from www.balaramaniam.com


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<b><a href="http://www.ncbi.nlm.nih.gov/htbin-
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Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;lffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.

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<b>Query=</b>
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<p>
<b>Database:</b> Sequences from complete mitochondrial genomes
      129 sequences; 3,164,247 total letters

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<p> <p>If you have any problems or questions with the results of this search
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	(bits)	Value
Sequences producing significant alignments:		

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```

```

<PRE>
  Database: Sequences from complete mitochondrial genomes
  Posted date: Jun 28, 2000 10:56 AM
  Number of letters in database: 3,164,247
  Number of sequences in database: 129

```

```

Lambda      K      H
1.37      0.711    1.31

```

```

Gapped
Lambda      K      H
1.37      0.711    1.31

```

```

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 788
Number of Sequences: 129
Number of extensions: 788
Number of successful extensions: 168
Number of sequences better than 10.0: 77
length of query: 328
length of database: 3,164,247
effective HSP length: 15
effective length of query: 313
effective length of database: 3,162,312
effective search space: 989803656
effective search space used: 989803656
T: 0
A: 30
X1: 6 (11.9 bits)
X2: 15 (29.7 bits)
S1: 12 (24.3 bits)
S2: 14 (28.2 bits)

```

```

</PRE>

```

```

</BODY>
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```

Table 4. Results of the blast analysis of the sequence revealed from ‘adil.flesh’ in ‘nr’ database of NCBI. It shows the most significant alignment of cytochrome b sequence (328 bp) revealed from confiscated skin piece ‘adil.flesh’ with *Panthera pardus* cytochrome b gene sequence (genbank registration number AY005809, bits score 603, E value, e-170) registered in NCBI database. It gives an indication that the species of analyzed material belongs to *Panthera pardus* origin. It also fulfills the requirements of column 6 mention above under sub-heading ‘Objectives of invention’.

Table 4. Results of the blast analysis of the sequence revealed from ‘adil.flesh’ in ‘nr’ database of NCBI.

```

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QBLASTInfoEnd
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<BR><BR><PRE>
<b>BLASTN 2.1.2 [Nov-13-2000]</b>

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```

<b><a href="http://www.ncbi.nlm.nih.gov/htbin-
post/Entrez/query?uid=9254694&form=6&db=m&Dopt=r">Reference</a>:</b>
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.

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<p>
<b>Query=</b>
(328 letters)

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```

<p>
<b>Database:</b> nt
      807,597 sequences; 2,863,827,885 total letters

```

```

<p> <p>If you have any problems or questions with the results of this search
<br>please refer to the <b><a
href=http://www.ncbi.nlm.nih.gov/blast/blast_FAQs.html>BLAST FAQs</a></b><br><p>
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Gapped
Lambda      K      H
1.37      0.711      1.31

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Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
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Table 5. Reference animal belonging to family felidae selected for comparison with ‘adil.flesh’ to confirm the findings of BLAST analysis, results of which are mentioned in Table 3 and 4, respectively. The animals listed in SN. 1-21 represent different species of family felidae. SN. 22 and 23 are primate species taken for out-group comparisons. The samples started with the code ‘bhz’ were collected from Bhuvaneshwar zoo, the samples with code numbers ‘gz’ from Guwahati zoo, samples coded with the number ‘darz’ from Darjeeling zoo, and the samples coded as ‘sbz’ were collected from Sakkarbaug zoo, India.

152

Table 5. Reference animals and the allocated code numbers included in the study

SN.	Code number	Name of the animal	Zoological name
1	bhz25t	Indian tiger	<i>Panthera tigris tigris</i>
2	bhz26t	Indian tiger	<i>Panthera tigris tigris</i>
3	bhz30t	Indian tiger	<i>Panthera tigris tigris</i>
4	bhz45t	Indian tiger	<i>Panthera tigris tigris</i>
5	bhz56t	Indian tiger	<i>Panthera tigris tigris</i>
6	bhz63t	Indian tiger	<i>Panthera tigris tigris</i>
7	bhz20wt	Indian white tiger	<i>Panthera tigris bengalensis</i>
8	bhz22wt	Indian white tiger	<i>Panthera tigris bengalensis</i>
9	bhz23wt	Indian white tiger	<i>Panthera tigris bengalensis</i>
10	bhz28wt	Indian white tiger	<i>Panthera tigris bengalensis</i>
11	gz1l	Normal leopard	<i>Panthera pardus</i>
12	gz2l	Normal leopard	<i>Panthera pardus</i>
13	gz3l	Normal leopard	<i>Panthera pardus</i>
14	gz21cl	Clouded leopard	<i>Neofelis nebulosa</i>
15	gz22cl	Clouded leopard	<i>Neofelis nebulosa</i>
16	darz14sl	Snow leopard	<i>Panthera unicia</i>
17	darz15sl	Snow leopard	<i>Panthera unicia</i>
18	darz16sl	Snow leopard	<i>Panthera unicia</i>
19	sbz22al	Asiatic lion	<i>Panthera leo persica</i>
20	sbz38al	Asiatic lion	<i>Panthera leo persica</i>
21	sbz39al	Asiatic lion	<i>Panthera leo persica</i>
22	humsk	Human	<i>Homo sapiens sapiens</i>
23	chimss	Chimpanzee	<i>Pan troglodytes</i>

Table 6. Multiple sequence alignments of cytochrome b sequences (328 bp) revealed from ‘adil.flesh’ and reference animals listed in Table 5. The positions that have a common nucleotide in all the animal species under investigation are shown with a star (*) mark; however, the positions that are variable in any of the animals under investigation are unmarked. The nucleotides at these positions constitute the molecular signature of an individual species, which are unique and highly specific for its species. These signatures are the molecular basis of identification of individual animal species using our primers ‘mcb398’ and ‘mcb869’.

Table 6

Table 6. Multiple sequence alignments of the cytochrome b sequences of reference animals with the sequence obtained from confiscated animal remain

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sbz38al	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
sbz39al	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
adil.flesh	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCCTGACACGATTCTTTGCCTTCCAC	60
gz1nl	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCCTGACACGATTCTTTGCCTTCCAC	60
gz2nl	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCCTGACACGATTCTTTGCCTTCCAC	60
gz3nl	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCTACCCTGACACGATTCTTTGCCTTCCAC	60
bhz23wt	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
bhz28wt	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
bhz22wt	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
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bhz63t	TGAATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCTTCCAC	60
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chimss	TGAATCTGAGGAGGCTTCTCAGTAGACAGCCCTACCCTTACACGATTCTTTACCTTCCAC	60
humsk	TGAATCTGAGGAGGCTTCTCAGTAGACAGTCCCACCCTCACACGATTCTTTACCTTTCAC	60

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sbz39al	TTCATCCTTCCATTTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTGTTCCCTCCATGAA	120
adil.flesh	TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCTTCCACGAG	120
gz1nl	TTCATCCTTCCATTTATCATCTCAGCTCTAGCAGCAGTCCACCTCCTATTCTTCCACGAG	120
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chimss	TTTATCTTACCCTTCATTATCACAGCCCTAACAACACTTCATCTCCTATTCTTACACGAA	120
humsk	TTCATCTTGCCTTCATTATTGCAGCCCTAGCAGCACTCCACCTCCTATTCTTGCACGAA	120
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gz2n1 ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATTCCATTCCACCCA 180
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bhz63t ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
bhz56t ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
bhz26t ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
bhz30t ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
bhz45t ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
bhz25t ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
dz14s1 ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
dz15s1 ACAGGATCTAACAACCCCTCAGGAATAGTATCTGACTCAGACAAAATCCCGTTCACCCA 180
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gz21c1 ACAGGATCCAATAACCCCTCAGGAATGGTATCCGATTAGACAAAATCCCGTTCACCCG 180
gz22c1 ACAGGATCCAATAACCCCTCAGGAATGGTATCCGATTAGACAAAATCCCGTTCACCCG 180
chimss ACAGGATCAAATAACCCCTGGGAATCACCTCCCACTCCGACAAAATTACCTTCCACCCC 180
humsk ACGGGATCAAACAACCCCTAGGAATCACCTCCCATTCGGATAAAATCATCTTCCACCCT 180
** * * * * *

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sbz39a1 TACTATACAATCAAAGATATCCTAGGCCTTCTAGTACTAATCTTAACACTCATACTACTC 240
adil.flesh TACTACACAATCAAAGATATCCTGGGCCTTCTAGTACTAATCCTAGCACTCATACTACTC 240
gz1n1 TACTACACAATCAAAGATATCCTGGGCCTTCTAGTACTAATCCTAGCACTCATACTACTC 240
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gz3n1 TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCTTAGCACTCATACTACTC 240
bhz23wt TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
bhz28wt TACTACACAATCAAAGACATCCTGGGCCTTCTAGTACTAATCCTAACACTCATACTACTC 240
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gz22c1 TACTATACAATCAAAGATATCCTAGGCCTCCTAGTTCTAATTCTAGCGCTCACACTACTT 240
chimss TACTACACAATCAAAGATATCCTTGGCTTATTCCTTTTCCTCCTTATCCTAATGACATTA 240
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bhz22wt    GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
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bhz63t     GTCCTATTCTCACCAGACCTATTAGGGGACCCCGATAACTACATCCCCGCCAACCCTCTA 300
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dz16sl     GTCCTATTCTCACCAGACCTATTAGGGGACGCCGATAACTACATCCCCGCCAACCCTCTA 300
gz21cl     GTTCTATTCTCCCCAGACCTACTAGGAGACCCTGACAATTACACTCCCGCCAACCCTCTA 300
gz22cl     GTTCTATTCTCCCCAGACCTACTAGGAGACCCTGACAATTACACTCCCGCCAACCCTCTA 300
chimss     ACACTATTCTCACCAGACCTCCTGGGCGATCCAGACAACCTATACCCTAGCTAACCCCTTA 300
humsk      ACACATTCTCACCAGACCTCCTAGGCGACCCAGACAATTATACCCTAGCCAACCCTTA 300
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sbz38al    AGCACCCCTCCCCATATCAAACCTGAAT 328
sbz39al    AGCACCCCTCCCCATATCAAACCTGAAT 328
adil.flesh AATACCCCTCCCCATATCAAGCCTGAAT 328
gz1nl      AATACCCCTCCCCATATCAAGCCTGAAT 328
gz2nl      AATACCCCTCCCCATATCAAGCCTGAAT 328
gz3nl      AATACCCCTCCCCATATCAAGCCTGAAT 328
bhz23wt    AACACCCCTCCCCATATCAAGCGCGAAT 328
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dz14sl     AACACCCCTCCCCATATCAAGCCCGAAT 328
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dz16sl     AACACCCCTCCCCATATCAAGCCCGAAT 328
gz21cl     AATACCCCTCCCCATATCAAGCCTGAAT 328
gz22cl     AATACCCCTCCCCATATCAAGCCTGAAT 328
chimss     AACACCCCAACCCACATTAACCCGAAT 328
humsk      AACACCCCTCCCCACATCAAGCCCGAAT 328
          * * * * * * * * * * * * *
```

Table 7 (Tables 7a, 7b, 7c and 7d). The comparison of the molecular signatures of different animal species investigated along with ‘adil.flesh’, the confiscated skin of unknown animal origin. This table demonstrates the variable positions (i.e. the positions which are not marked with star (*) symbol in Table 6), amongst the 328 bp fragment revealed from the animals listed in Table 5. The dot (.) mark represents the presence of the similar nucleotide as listed in lane 1 i.e. the sequence from “adil.flesh” at that position. It demonstrates that the signatures of each species are unique and specific to its species. The molecular signatures of ‘adil.flesh’ are comparable (except for position 37 which has a transition from ‘T’ to ‘C’) to the molecular signature of ‘gz1L’ i.e. the known leopard ‘*Panthera pardus*’ source, indicating the identity of the source of confiscated skin ‘adil.flesh’ as that of a leopard ‘*Panthera pardus*’ source. The nucleotide variations (at the positions 153, 198, 223, 264, among the known leopards, (i.e. gz1L, gz2L, and gz3L, respectively)), give an idea about the geographical habitat of each animals. Various studies referring to molecular evolution of different animal species support this hypothesis⁷⁵; however, it could further be confirmed by taking the reference animals from different geographical areas and analyzing by our primers ‘mcb 398’ and ‘mcb869’. If we could generate the database of different haplotypes (i.e. habitat specific molecular signatures) of the animal species, it would also enable our primers to reveal the geographical location of the commitment of wildlife crime.

Table 7a

Position	17	25	29	30	31	33	37	39	48	51	52	57	63	67	69	72	75	78	81	82	87	88	91	94	97	99	102	105	108	111	112
adil.flesh	T	G	A	A	G	T	C	G	C	T	G	C	C	C	T	A	T	C	C	T	T	C	G	G	G	C	C	C	A	C	C
gz1l	T
gz2l	T
gz3l	T
bhz25t	C	C
bhz26t	C	C
bhz30t	C	C
bhz45t	C	C
bhz56t	C	C
bhz20wt	C	C
bhz22wt	C	C
bhz23wt	C	C
dz14sl	.	C	.	.	.	C	C
dz15sl	.	C	.	.	.	C	C
sbz22al	C	C
sbz38al	C	C
gz21cl	C	.	.	T	C	C	T	T	T	T	T	T
gz22cl	C	.	.	T	C	C	T	T	T	T	T	T
chimss	A	.	G	C	C	.	.	T	.	C	A	T	T	T	A	C	C	T	.	A	C	.	A	A	C	C	T	.	.	.	T
humsk	A	.	G	T	C	C	A	T	T	T	G	C	C	T	T	G	C	T

Table 7b

Position	114	117	120	123	129	132	139	140	141	147	148	149	150	153	154	156	159	162	168	169	170	171	177	180	186	198	199	200	204	208	210	
adil.flesh	T	C	G	A	T	C	T	C	A	A	G	T	A	C	G	C	A	C	T	C	C	A	C	A	C	T	A	T	G	C	T	
gz1l
gz2l
gz3l
bhz25t	C	T	T
bhz26t	C	T	T
bhz30t	C	T	T
bhz45t	C	T	T
bhz56t	C	T	T
bhz20wt	C	T	T
bhz22wt	C	T	T
bhz23wt	C	T	T
dz14sl	C	T	T
dz15sl	C	T	T
sbz22al	C	T	T	A	G
sbz38al	C	T	T	A	G
gz21cl	C	T	T	A	G
gz22cl	C	T	T	A	G
chfms	A	.	A	A	A	T	C	T	G	C	A	C	C	.	C	T	C	T	C	A	T	C
humsk	G	.	A	G	A	.	C	T	.	C	A	C	C	.	C	T	C	T	C	A	T	C

Table 7c

Position	211	213	214	216	217	219	220	222	223	225	226	227	228	229	231	233	234	235	236	238	240	241	242	243	252	261	262	264	267	270	271
adl.flesh	C	A	G	A	C	A	A	C	C	A	G	C	A	C	C	T	A	C	T	C	G	G	T	C	A	G	T	A	A	C	C
gz1l
gz2l
gz3l
bhz25t	A	A	A	.	.	G	.	.
bhz26t	A	A	A	.	.	G	.	.
bhz30t	A	A	A	.	.	G	.	.
bhz45t	A	A	A	.	.	G	.	.
bhz56t	A	A	A	.	.	G	.	.
bhz20wt	A	A	A	.	.	G	.	.
bhz22wt	A	A	A	.	.	G	.	.
bhz23wt	A	A	A	.	.	G	.	G
dz14sl	A	A	A	.	.	G	.	G
dz15sl	A	A	A	.	.	G	.	G
sbz22al	A	A	A
sbz38al	A	A	A
gz21cl	A	A	A
gz22cl	A	A	A
chmiss	T	C	C	T	T	C	C	T	T	T	A	T	C	A	A	G	A	C	T	A	A	A	C	A	A	C	C	C	G	C	T
humsk	T	C	C	T	T	C	C	T	T	T	A	T	C	A	A	G	A	C	T	A	A	A	C	A	A	C	C	C	G	C	T

Table 7d

Position	273	276	279	282	284	285	287	288	291	294	297	298	302	303	309	315	318	321	323	324
adil.flesh	C	T	C	C	T	C	C	T	C	C	T	C	A	T	T	T	C	G	C	T
gz1l
gz2l
gz3l
bhz25t	G	C
bhz26t	G	C
bhz30t	G	C
bhz45t	G	C
bhz56t	G	C
bhz20wt	G	C
bhz22wt	G	C
bhz23wt	G	C
dz14sl
dz15sl
sbz22al	.	C	.	T	C	.	.	C	.	T	.	.	G	C	.	.	.	A	.	.
sbz38al	.	C	.	T	C	.	.	C	.	T	.	.	G	C	.	.	.	A	.	.
gz21cl	T	C	T	.	C	T	.	C
gz22cl	T	C	T	.	C	T	.	C
chmss	A	C	T	T	C	.	T	A	T	.	C	.	.	C	A	C	T	A	.	C
humsk	A	C	T	T	C	.	T	A	.	.	C	.	.	C	.	C	.	.	.	C

Table 8. Percent similarity matrix calculated by pair-wise comparisons of nucleotide sequences aligned (illustrated in Table 6). The cytochrome b gene sequence of DNA isolated from confiscated material had maximum similarity (99.7% and 98.2%, with the lineages of animals 'gz2L' and 'gz3L', respectively) with the sequences obtained from known normal leopard source, indicating its identity as that of a leopard origin. The similarity matrix has been calculated using the software *PHYLIP* (3.5).

4.0000 0.9970 0.9820

Table 8. Percent similarity matrix calculated by pair-wise comparisons of cytochrome b gene sequences revealed from 'adil.flesh' and different felids

	bhz20wt	bhz25t	dz14sl	humsk	chimss	sbz22al	gz1L	gz2L	gz3L	gz21ci	adil.flesh
bhz20wt		100	99.1	81.7	78.7	93.3	95.1	95.4	95.4	89.6	95.4
bhz25t	100		99.1	81.7	78.7	93.3	95.1	95.4	95.4	89.6	95.4
dz14sl	99.1	99.1		81.4	78.4	93	94.8	95.1	95.1	89.3	95.1
humsk	81.7	81.7	81.4		86.9	79.6	81.1	80.2	80.2	79	81.4
chimss	78.7	78.7	78.4	86.9		78.7	79.6	78.7	78.7	76.8	79.9
sbz22al	93.3	93.3	93	79.6	78.7		92.1	92.4	92.4	89	92.4
gz1L	95.1	95.1	94.8	81.1	79.6	92.1		98.5	98.5	89.3	99.7
gz2L	95.4	95.4	95.1	80.2	78.7	92.4	98.5		100	88.1	98.2
gz3L	95.4	95.4	95.1	80.2	78.7	92.4	100	88.1		88.1	98.2
gz21ci	89.6	89.6	89.3	79	76.8	89	88.1	88.1	88.1		89.6
adil.flesh	95.4	95.4	95.1	81.4	79.9	92.4	99.7	98.2	98.2	89.6	

Table 9. Animals selected for validation of minimum P,S score for efficient amplification of cytochrome b gene of different origin by the primers ‘mcb398’ and ‘mcb869’. P,S score of primers ‘AFF’ and ‘AFR’ for these animals are shown.

Accepted for publication

Table 9. Animals selected for validation of minimum P'S score for efficient amplification of DNA templates in PCR

SL.	Name	P, S/AFF	P, S/AFR
1	Indian black buck (<i>Antelope cervicapra</i>)	97, 58	96, 54
2	Sheep (<i>Ovis</i>)	87, 53	96, 54
3	Pig (<i>Sus scrofa</i>)	87, 52	87, 41
4	Fresh water dolphin (<i>Platanista gangetica</i>)	86, 49	82, 47

Table 10. BLAST analysis of primers ‘mcb398’ in *nr* database of NCBI . It demonstrates that the 3’ end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer

Table 10. BLAST analysis of primers ‘mcb398’ in *nr* database of NCBI .

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Sequences producing significant alignments:

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platyrhinos cytochrome b ... 50 2e-05
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 cytochrome b (cytb) g... 50 2e-05
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 contortrix cytochrome b ... 50 2e-05
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 [sheep, domestic, Merino... 50 2e-05
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 cytochrome b gene, co... 50 2e-05
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 jugossicularis cyto... 50 2e-05
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 cruentus cytochrome b (cyt... 50 2e-05
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gb|AF022063.1| Tragelaphus strepsiceros cytochrome b (cytb)... 50 2e-05
gb|AF022062.1| Tragelaphus derbianus cytochrome b (cytb) ge... 50 2e-05
gb|AF022060.1| Hippotragus equinus cytochrome b (cytb) gene... 50 2e-05
gb|AF022057.1| Tragelaphus oryx cytochrome b (cytb) gene, m... 50 2e-05
gb|AF113500.1|AF113500 Lagenorhynchus acutus isolate LACU94... 50 2e-05
gb|AF113499.1|AF113499 Lagenorhynchus acutus isolate LACU93... 50 2e-05
gb|U69845.1|LBU69845 Loxocemus bicolor cytochrome b (cytb) ... 50 2e-05
gb|U69810.1|ENU69810 Eunectes notaeus cytochrome b (cytb) g... 50 2e-05
gb|U69808.1|EMU69808 Eunectes murinus cytochrome b (cytb) g... 50 2e-05
gb|U69799.1|ESU69799 Epicrates striatus fosteri cytochrome ... 50 2e-05
gb|U69796.1|ESU69796 Epicrates striatus strigilatus cytochr... 50 2e-05
gb|U69795.1|ESU69795 Epicrates striatus strigilatus cytochr... 50 2e-05
gb|U69794.1|ESU69794 Epicrates striatus mcraniei cytochrom... 50 2e-05
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cytochrome b (cytb) ge... 50 2e-05
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cytochrome b gene,... 50 2e-05
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mitochondrion, comple... 50 2e-05
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tigris strain Isla Ang... 50 2e-05
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e&list_uids=04565823&dopt=GenBank">gb|AF006267.1|AF006267 Cnemidophorus
tigris strain Isla Smi... 50 2e-05
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href="http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=Nucleotid
e&list_uids=02660921&dopt=GenBank">gb|AF034969.1|AF034969 Connochaetes
taurus cytochrome b g... 50 2e-05
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buselaphus cytochrome b g... 50 2e-05
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lunatus cytochrome b gene... 50 2e-05
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jamaicensis mitochondrial D... 50 2e-05
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impavida cytochrome b (... 50 2e-05
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carteri cytochrome b (c... 50 2e-05
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furcata cytochrome b (cy... 50 2e-05
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 pelagicus cytochrome b (c... 50 2e-05
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 cytochrome b (cytb) ... 50 2e-05
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carlinianus mitochondrion ... 50 2e-05
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crispus mitochondrial ge... 50 2e-05
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mitochondrial gene for ... 50 2e-05
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mitochondrial gene for cytochr... 50 2e-05
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americanus mitochondrial ge... 50 2e-05
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mitochondrial gene for... 50 2e-05
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scoticus mitochondri... 50 2e-05
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canadensis mitochond... 50 2e-05
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nippon mitochondrial ... 50 2e-05
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dbj|AB008539.1|AB008539 Dinodon semicarinatus mitochondrial... 50 2e-05
dbj|AB006800.1|AB006800 Ovis aries mitochondrial DNA for cy... 50 2e-05
gb|L12763.1|LDHMTCTB Lepidochelys kempi (LK-3) mitochondri... 50 2e-05
gb|L08032.1|CPLMTCYTBA Carcharhinus plumbeus mitochondrial ... 50 2e-05
gb|L28941.1|URRCYB Uroderma bilobatum cytochrome b gene, 5'... 50 2e-05
gb|L28937.1|CDECYB Chiroderma doriae cytochrome b gene, 5' end 50 2e-05
emb|AJ010056.1|CPY010056 Capra pyrenaica (individual 12) mi... 50 2e-05
emb|AJ010054.1|CPY010054 Capra pyrenaica (individual 11) mi... 50 2e-05
emb|AJ010053.1|CPY010053 Capra pyrenaica (individual 10) mi... 50 2e-05
emb|AJ010052.1|CPY010052 Capra pyrenaica (individual 9) mit... 50 2e-05
emb|AJ010051.1|CPY010051 Capra pyrenaica (individual 8) mit... 50 2e-05
emb|AJ010050.1|CPY010050 Capra pyrenaica (individual 7) mit... 50 2e-05
emb|AJ010049.1|CPY010049 Capra pyrenaica (individual 6) mit... 50 2e-05
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Number of letters in database: 2,863,827,885
Number of sequences in database: 807,597

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1.37	0.711	1.31

Gapped

Lambda	K	H
1.37	0.711	1.31

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 39355
Number of Sequences: 807597
Number of extensions: 39355
Number of successful extensions: 15066
Number of sequences better than 10.0: 5706
length of query: 25
length of database: 2,863,827,885
effective HSP length: 17
effective length of query: 8
effective length of database: 2,850,098,736
effective search space: 22800789888
effective search space used: 22800789888
T: 0
A: 30
X1: 6 (11.9 bits)
X2: 15 (29.7 bits)
S1: 12 (24.3 bits)
S2: 16 (32.2 bits)

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Table 11. BLAST analysis of primers ‘mcb869’ in *nr* database of NCBI. It demonstrates that the 3’ end of this primer is highly conserved among a vast range of animal species. It also shows the significant homology among the primer and templates (i.e. the cytochrome b gene fragment of different animal species), confirming the universal nature of our primer.

Table 11. BLAST analysis of primers ‘mcb869’ in *nr* database of NCBI.

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<b><a href="http://www.ncbi.nlm.nih.gov/htbin-
post/Entrez/query?uid=9254694&form=6&db=m&Dopt=r">Reference</a>:</b>
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Sch&auml;ffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.

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<p> <p>If you have any problems or questions with the results of this search
<br>please refer to the <b><a
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Sequences producing significant alignments:

	Score (bits)	E Value
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Gapped

Lambda	K	H
1.37	0.711	1.31

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Number of extensions: 19068
Number of successful extensions: 7580
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Table 12. Other animal belonging to distantly related animal species, investigated to confirm the universal nature of primers ‘mcb398’ and ‘mcb869’. Gel photograph showing the PCR amplicons from these animals are shown in Figure 4.

Table 12. Other animal belonging to distantly related animal species, investigated to confirm the universal nature of primers ‘mcb398’ and ‘mcb869’. Gel photograph showing the PCR amplicons from these animals are shown in Figure 4.

Table 12. The other animals belonging to distantly related species analyzed by our primers to demonstrate its universal nature

SN.	Name of the animal
1.	Indian black buck no.1
2.	Indian black buck no 2
3	sheep
4	pig
5	dog
6	chimpanzee (chimss)
7	human (humsk)
8	Hamster
9	crocodile no1
10	crocodile no2
11	turtle no1
12	turtle no2
13	mouse
14	varanus
15	Naga-naga snake
16	Indian elephant
17	hen
18	dugong
19	lizard
20	weaver bird no1
21	weaver bird no2
22	buffalo no1
23	buffalo no 2